Transcriptional control of the $\alpha 1(I)$ collagen gene involves orientation- and position-specific intronic sequences

Anne L. SHERWOOD* and Paul BORNSTEIN†

Departments of Biochemistry and Medicine, University of Washington, Seattle, WA 98195, U.S.A.

The 3' half of the first intron of the human $\alpha 1(I)$ collagen gene interacts with the promoter to regulate transcription. We questioned whether this intronic sequence also exerted its effect when placed 5' to the promoter. In transient transfection assays using several cell lines, little or no stimulation of $\alpha 1(I)$ -driven chloroamphenicol acetyltransferase transcription was observed. We conclude that transcriptional control of the $\alpha 1(I)$ gene is dependent on a complex series of interactions that require both orientation and position specificity of the intronic segment.

INTRODUCTION

A number of studies have implicated the first intron in the transcriptional regulation of the $\alpha 1(I)$ collagen gene [1–5]. Rossouw *et al.* [5] first reported that a 786 bp endonuclease-*Sma*I fragment, comprising approximately the 3' half of the first intron, markedly enhanced expression of chimaeric collagen- α -globin gene plasmids when introduced by microinjection into *Xenopus* oocytes. Enhancement was seen when the intronic fragment was placed in either orientation in the first intron of the globin gene, driven by a short (to –253) collagen promoter; a 100-fold enhancement was also observed when a similar construct was introduced by DNA transfection into NIH-3T3 cells. When the same intronic fragment was cloned in the positive orientation, 5' to a longer (to –950) collagen promoter, an 18-fold enhancement was seen in *Xenopus* oocytes, but no effect was noted in the negative orientation.

Our own work has not produced findings in total agreement with those mentioned above. An intronic fragment of 550 bp that partially overlapped the 786 bp fragment used by Rossouw *et al.* [5] was neutral when cloned 5' to a collagen promoter–chloramphenicol acetyltransferase (CAT) gene and tested in chicken tendon fibroblasts (CTF); the same fragment produced a 2-fold reduction in CAT activity in the negative orientation [1]. Furthermore, inverting a longer intronic segment that included most of the 786 bp *Sma*I fragment in its natural position 3' to the promoter led to a marked inhibition of transcription of collagen–human growth hormone (hGH) chimaeric plasmids introduced into CTF [2]. Rippe *et al.* [4] also found that the predominant effect of sequences in the mouse $\alpha 1(I)$ intron was inhibitory when these were cloned 3' to the CAT gene in mouse promoter–CAT constructs and transfected into NIH-3T3 cells.

Although some of these findings appear to be contradictory, there are differences in experimental detail that could be important: (a) intronic sequences were placed in different positions relative to the promoter; (b)

the same intronic segments were not used in all experiments; (c) collagen promoters of different length were used; and (d) DNA was introduced into different cells. Because we have advanced a model for the role of the

Fig. 1. Structure of plasmid pCol-CAT and its derivatives

The CAT gene, driven by an $\alpha 1(I)$ collagen promoter, extending from –331 (*Xba*I) to +97 (*Acc*I), was inserted into pUC18. A 786 bp *Sma*I fragment extending from 822 to 1607 in the first intron of the $\alpha 1(I)$ gene was inserted, in either orientation, into the blunt-ended *Bam*HI site. All numbers are relative to the start of transcription in the collagen gene (taken as 1). The CAT sequence contains, in addition to the CAT structural gene, the small t intron and a poly(A) signal [11].

Abbreviations used: CAT, chloramphenicol acetyltransferase; CTF, chicken tendon fibroblasts; hGH, human growth hormone; pCol, collagen promoter (in the plasmid pCol-CAT); HFF, human foreskin fibroblasts; SV-MSC, simian-virus-transformed bone-marrow stromal cells; RSV-LTR, Rous-sarcoma-virus long terminal repeat.

* Present address: Pacific NW Research Foundation, Department of Biochemical Oncology, 720 Broadway, Seattle, WA 98122, U.S.A.

† To whom correspondence and reprint requests should be sent.

first intron in the regulation of transcription of the human $\alpha 1(I)$ collagen gene [2], we thought it important to determine whether we could confirm the existence of an enhancer of the type proposed by Rossouw *et al.* [5] in the 3' half of the first intron.

EXPERIMENTAL

We modified a previously described plasmid, pCol-CAT [3] (where 'pCol' refers to the collagen promoter), by the introduction of an *SmaI* fragment, extending from 822 to 1607 in the first intron of the human $\alpha 1(I)$ gene, into a blunt-ended *Bam*HI site 5' to the collagen promoter (Fig. 1). This fragment is identical with that used by Rossouw *et al.* [5] in their studies of the human $\alpha 1(I)$ gene. In addition, a similarly sized *SmaI* fragment, located between -800 and -2300 in the $\alpha 1(I)$ gene, was cloned into the same blunt-ended site. In order to assess the effect of promoter length on the ability of the intronic fragment to stimulate transcription, we obtained from Dr. S. Boast and Dr. F. Ramirez (SUNY

Downstate Medical Center, Brooklyn, NY, U.S.A.) analogous CAT-based plasmids in which the intronic *SmaI* fragment was cloned 5', in either orientation, to a collagen promoter extending from -804 (*Hind*III) to +114 (*Xba*I).

Plasmids were introduced by the calcium phosphate co-precipitation technique [3,6] into (a) human foreskin fibroblasts (HFF), (b) a human fibroblast-like line derived by simian-virus-40 transformation of bone-marrow stromal cells (SV-MSC [7]), (c) CTF and (d) NIH-3T3 cells. Efficiency of uptake of DNA was monitored by co-transfection with (a) a plasmid containing the β -galactosidase gene driven by the Rous-sarcoma-virus long terminal repeat (RSV-LTR), (b) an RSV-LTR-luciferase plasmid [8] or (c) a metallothionein I-hGH plasmid [9]. Results were comparable when any of the three transfection control plasmids was used. CAT activity was measured primarily by a non-chromatographic phase-partition assay [10], but values obtained with the conventional chromatographic separation of acetylated chloramphenicol [6] were comparable.

pCol-CAT plasmid	Intron	Promoter	CAT	Relative CAT activity			
				HFF	SV-MSC	CTF	NIH-3T3
1		-331 97		1.0	1.0	1.0	1.0
2	822 1607			2.0 ± 0.81 (3)	1.2 ± 0.30 (4)	1.2 ± 0.26 (5)	0.70 (0.41; 0.98)
3	1607 822			1.4 ± 1.0 (3)	1.3 ± 0.42 (4)	0.35 ± 0.01 (5)	0.47 ± 0.17 (3)
4	5' Flank			2.1 ± 1.0 (3)	0.78 ± 0.17 (3)	1.2 ± 0.14 (5)	1.6 ± 1.1 (3)
5		-804 114			0.86 ± 0.19 (4)	0.77 (0.43; 1.1)	
6	822 1607				0.84 ± 0.26 (4)	0.45 (0.2; 0.7)	
7	1607 822				0.74 ± 0.22 (4)	0.37 (0.31; 0.43)	
8		SV2		2.0 ± 0.51 (3)	9.5 (18.0; 0.9)	2.3 ± 0.88 (5)	2.2 ± 0.65 (3)
9		pUC18			0.056 ± 0.01 (3)		

Fig. 2. Relative CAT activity of pCol-CAT plasmids, introduced by calcium phosphate-mediated transfection, into HFF, SV-MSC, CTF and NIH-3T3 cells

All values represent the mean $\pm$ S.E.M., with the number of independent determinations (or individual values where $n = 2$) in parentheses. Values were corrected for efficiency of transfection, except for data reported for CTF. In these cells the reproducibility of transfection was judged to be better than that provided by the use of a co-transfected β -galactosidase plasmid. However, corrected values were not significantly different. The open arrow signifies the orientation of the collagen intronic fragment, extending from base 822 to base 1607. On line 4 the 5' flank sequence is a similarly sized *SmaI* fragment located between -800 and -2300 in the $\alpha 1(I)$ gene. The SV2-CAT plasmid contains both the 72 bp enhancer and 21 bp repeat promoter [6].

RESULTS AND DISCUSSION

The results of our experiments are summarized in Fig. 2. There was little or no effect of the 786 bp *Sma*I intronic fragment when placed 5' to a short (to -331) collagen promoter and introduced into either HFF or SV-MSC cells (Fig. 2; constructs 1-4). The 2-fold stimulation observed with the 786 bp fragment in the positive orientation in HFF was no greater than that seen with a similarly sized fragment derived from the 5' flanking sequence of the $\alpha 1(I)$ gene. In CTF, and possibly in NIH-3T3 cells, there was a modest (2-fold) inhibition of CAT activity, but only when the fragment was placed in the negative orientation (Fig. 2; construct 3). The same results were obtained by us in CTF using a 552 bp intronic fragment extending from nucleotide 855 to nucleotide 1407 [1]. We did not observe the 18-100-fold stimulation (depending on position, orientation and length of promoter) reported by Rossouw *et al.* [5] for the 786 bp intronic fragment. pSV2-CAT [6] yielded CAT activity that was only about 2-fold higher than pCol-CAT (Fig. 2; construct 8), since the collagen promoter is highly active in fibroblast-like cells. A similar observation was made by Rippe *et al.* [4]. A promoterless CAT construct produced CAT activities that were only slightly above background (Fig. 2; construct 9).

Since the ability of the 786 bp intronic fragment to serve as an enhancer could depend on the length of the collagen promoter with which it presumably interacts, we also tested the transcriptional activity of plasmids containing a collagen promoter that extends to -804 (Fig. 2; constructs 5-7). The addition of the intronic fragment in either orientation had essentially no effect in SV-MSC cells. In CTF a modest (2-fold) inhibitory effect may exist, but more determinations will be required to substantiate this result. It should be noted that other investigators have observed a significant stimulation in CAT activity when constructs 6 and 7 (Fig. 2) were transiently expressed in HFF or in adult human lymphoblasts (F. Ramirez, personal communication). We do not have a good explanation for this discrepancy. However, results identical with ours were obtained with chick-embryo fibroblasts.

We conclude, on the basis of our experiments, that a 'classical' enhancer of the type proposed by Rossouw *et al.* [5], i.e. an element that strongly activates transcription and is relatively independent of orientation and position, does not exist in the 3' half of the first intron of the

human $\alpha 1(I)$ gene. Our results are more consistent with the data of Rippe *et al.* [4], who were also unable to detect a strongly positively acting element in this region of the first intron of the mouse $\alpha 1(I)$ gene. Nevertheless, we believe that this region of the intron is involved in transcriptional regulation of the gene, since there is orientation specificity of these sequences *in situ* and there is evidence for interaction with the promoter [2]. The findings reported here are consistent with an emerging picture of a complex interplay of multiple intronic elements interacting with promoter sequences via DNA-binding proteins to effect transcriptional regulation of the $\alpha 1(I)$ collagen gene [2].

We are indebted to Dr. S. Boast and Dr. F. Ramirez for constructs 5-7 (Fig. 2) and Dr. J. L. Slack for the SV-MSC cells. We thank Ms. Kathleen Doehring for preparation of the manuscript, Mr. Tim Lane for preparation of the Figures and Dr. H. Sage and Dr. D. J. Liska for a critical reading of the paper. This work was supported by Public Health Service grants AM 11248, DE 08229 and HL 18645 from the National Institutes of Health.

REFERENCES

1. Bornstein, P. & McKay, J. (1988) *J. Biol. Chem.* **263**, 1603-1606
2. Bornstein, P., McKay, J., Liska, D. J., Apone, S. & Devarayalu, S. (1988) *Mol. Cell. Biol.* **8**, 4851-4857
3. Bornstein, P., McKay, J., Morishima, J. K., Devarayalu, S. & Gelinas, R. E. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8869-8873
4. Rippe, R. A., Lorenzen, S., Brenner, D. A. & Breindl, M. (1989) *Mol. Cell. Biol.* **9**, 2224-2227
5. Rossouw, C. M. S., Vergeer, W. P., duPlooy, S. J., Bernard, M. P., Ramirez, F. & de Wet, W. J. (1987) *J. Biol. Chem.* **262**, 15151-15157
6. Gorman, C. (1985) in *DNA Cloning, A Practical Approach*, vol. 2 (Glover, D. M., ed.), pp. 143-190, IRL, Oxford
7. Singer, J. W., Charbord, P., Keating, A., Nemunaitis, J., Raugi, G., Wight, T. N., Lopez, J. A., Roth, G. J., Dow, L. W. & Failkow, P. J. (1987) *Blood* **70**, 464-474
8. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7**, 725-737
9. Selden, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986) *Mol. Cell. Biol.* **6**, 3173-3179
10. Sleight, M. J. (1986) *Anal. Biochem.* **156**, 251-256
11. Gorman, C., Moffat, L. & Howard, B. (1982) *Mol. Cell. Biol.* **2**, 1044-1051

Received 31 October 1989; accepted 27 November 1989