Biochem. J. (2000) 347, 55–59 (Printed in Great Britain)


# PTRF (polymerase I and transcript-release factor) is tissue-specific and interacts with the BFCOL1 (binding factor of a type-I collagen promoter) zinc-finger transcription factor which binds to the two mouse type-I collagen gene promoters

Tadao HASEGAWA, Akihide TAKEUCHI, Osamu MIYAISHI, Hengyi XIAO, Jalin MAO and Ken-ichi ISOBE[1]

Department of Basic Gerontology, National Institute for Longevity Sciences, 36-3, Gengo Morioka-cho, Obu, Aichi, 474-8522 Japan

We have used the yeast two-hybrid system to clone the protein that interacts with the BFCOL1 (binding factor of a type-I collagen promoter) zinc-finger transcription factor that was cloned previously as the factor that binds to the two mouse proximal promoters of the type-I collagen genes. We utilized as bait the N-terminal domain of BFCOL1 that includes the zinc-finger DNA-binding domain. One cDNA contained a potential open reading frame for a polypeptide of 392 amino acids and was identical to PTRF (polymerase I and transcript-release factor), which is involved in transcription termination of the RNA polymerase I reaction. Northern-blot analysis revealed that the pattern of mRNA expression was similar to that of the type-I collagen gene. In addition, we detected the mRNA expression only in a fibroblast cell line and two bone cell lines, but not in other blood and neuronal cell lines. Recombinant protein was shown to enhance the binding of BFCOL1 to its binding site in the mouse pro$\alpha$2(I) collagen proximal promoter *in vitro*. The transient-transfection experiment showed that PTRF had a suppressive effect on the mouse pro$\alpha$2(I) collagen proximal promoter activity. We speculate that PTRF might play a role in the RNA polymerase II reaction as well as that of RNA polymerase I.

Key words: luciferase assay, RNA polymerase II, transcription termination, transcriptional suppressor, yeast two-hybrid system.

## INTRODUCTION

Type-I collagen is synthesized by a discrete number of cell types that include fibroblasts, osteoblasts, odontoblasts, smooth-muscle cells and mesenchymal cells. It is composed of two $\alpha$1 chains and one $\alpha$2 chain forming a characteristic triple helix. Expression of the genes for these polypeptides is co-ordinately regulated in a variety of physiological and pathological conditions [1]. Changes in the synthesis of type-I collagen occur not only during embryonic development in specific tissues but also under pathological conditions, for example, during wound healing and in fibrotic diseases such as lung fibrosis, cirrhosis and scleroderma. In many cases, it is likely that the control of expression of the two type-I collagen genes is mainly exerted at the level of transcription, but the precise mechanisms that control transcription of these genes are still poorly understood. Previous studies have identified several functional *cis*-acting elements in the 350-bp proximal promoter of the mouse *pro$\alpha$2*(I) collagen gene [2]. These include a binding site for the ubiquitous heterotrimeric CCAAT-binding factor (CBF) between $-75$ and $-98$ [3–5], and redundant GC-rich binding sites for several proteins between $-65$ and $-105$, between $-114$ and $-131$ and between $-152$ and $-176$ [6]. Transient expression and transcription experiments *in vitro* with wild-type and mutant templates indicated that three elements were essential for promoter activation [6]. The proteins that bind to these sites are mainly ubiquitous proteins, such as those of the Sp1 family and Krox family. In addition, an element that strongly enhanced the expression in fibroblasts and mesenchymal cells was shown to be located between 13.5 and 17.5 kb upstream of the transcription start site in the *pro$\alpha$2*(I) gene [7].

We speculate that proteins binding to the upstream enhancers in both type-I collagen genes co-operate with transcription factors binding to the proximal promoters to activate transcription in specific cell types. Among the ubiquitous proteins in the proximal promoter, we cloned the transcription factor, BFCOL1 (binding factor of a type-I collagen promoter) [8]. This transcription factor was expressed in all the tissues examined; however, it did not belong to either the Sp1 or the Krox transcription factor families. This transcription factor binds to the two type-I proximal promoters and the binding site was identical to the inhibitory factor (IF) 1-binding site that was shown previously to contain negatively regulated *cis*-elements [9,10]. In our previous study, we mentioned that BFCOL1 might act as a repressor; however, the specificity was not clear. Hence, the purpose of this study is to investigate further the mechanism of function of BFCOL1. For this purpose, we tried to clone the protein that interacts with BFCOL1, because many functional cofactors that interact with a transcription factor have been cloned so far. One cDNA that was cloned partially encodes a gene identical to PTRF (polymerase I and transcript-release factor), which is involved in the transcription of the RNA polymerase I reaction [11]. We named our cDNA BBP, a BFCOL1-binding protein. Here we show the results of experiments on BBP/PTRF and the transcription of the type-I collagen promoter.

## EXPERIMENTAL

### Cloning of a cofactor protein with BFCOL1 using the yeast two-hybrid system

The yeast strain Y190 (*MATa*, *leu2-3,112*, *ura3-52*, *trp1-901*, *his3*-$\Delta$200, *ade2-101*, *gal4*$\Delta$gal80$\Delta$ URA3 GAL1-lacZ, *LYS*

Abbreviations used: PTRF, polymerase I and transcript-release factor; BFCOL1, binding factor of a type-I collagen promoter; BBP, BFCOL1-binding protein; CBF, CCAAT-binding factor; IF, inhibitory factor; RACE, rapid amplification of cDNA ends; GST, glutathione S-transferase.

[1] To whom correspondence should be addressed (e-mail kenisobe@nils.go.jp).

© 2000 Biochemical Society

*GAL-HIS3*, *cyh*[r]) was purchased from Clontech. The yeast reporter plasmid was constructed as follows. A *Sac*I–*Sal*I fragment of the pAS2-1 vector (Clontech) containing the GAL4-binding domain was inserted into the *Sac*I–*Sal*I site of pRS305HIS [8,12] to generate pRS305HISpAS. The *Sma*I–*Nsi*I fragment of pPC86BFCOL1 [8] was subcloned into the *Sma*I–*Pst*I site of the pGBT9 vector (Clontech) to make pGBT9-BFCOL1N. The *Sma*I–*Nae*I fragment of pGBT9-BFCOL1N was subcloned in frame into the *Sma*I site of the pRS305HISpAS vector to make pRS305HISpAS-BFCOL1N that contained the *leu2* gene as a selectable marker. After digestion with *Cla*I, this vector was used for transformation.

Yeast transformation was performed by the polyethylene glycol/lithium acetate method [13]. Plasmid integration into the genomes of yeast strains was confirmed by Southern-blot analysis using a $^{32}$P-labelled *Sma*I–*Nsi*I fragment of pPC86BFCOL1. Cells were then plated on a minimal synthetic dextrose plate without histidine to verify background *his3* gene activity. One of the yeast strains that had minimal *his3* gene activity was selected as the strain for the transformation after the initial selection. The yeast strain in which pRS305HISpAS-BFCOL1N was integrated was used for cDNA library transformation. cDNA libraries constructed in the pPC86 vector were the same as those described previously [8]. cDNA plasmid (10 $\mu$g) from the libraries was transformed into the yeast strain harbouring the reporter plasmid integrated into the genome and plated on to plates containing 5 mM 3-amino-1,2,4-triazole, but lacking leucine, tryptophan and histidine. The transformation efficiency was about $1 \times 10^5/\mu$g of cDNA plasmid. Colonies were picked after 3–5 days. Plasmid cDNAs were extracted and used for retransformation either into the same yeast strain or into the yeast strain into which the pRS305HISpAS plasmid instead of the pRS305HISpAS-BFCOL1N plasmid had been integrated.

### DNA sequencing and generating the full-length BBP

DNA sequencing was carried out using a Pharmacia ALFDNA sequencer with M13 universal and reverse-sequence primers.

To generate the full-length BBP, rapid amplification of cDNA ends (RACE) was performed using the Clontech Marathon™ cDNA amplification kit. PCR products were subcloned into the pGEMTeasy vector (Promega).

### Expression of cloned cDNA by transcription and translation *in vitro*

Two different recombinant polypeptides corresponding to the partial original cDNA and full-length BBP were generated using the TNT-coupled reticulocyte lysate system (Promega). For the original BBP polypeptide, the *Eco*RI fragment of pPC86-BBPori was inserted into the *Eco*RI site of the pCITE2C vector (Novagen) to produce pCITE2C-BBPori. For the full-length polypeptides, the *Nco*I fragment of pGEMTeasy BBP was inserted into the *Nco*I site of pCITE2C vector (pCITE2C-BBPfull). $^{35}$S-Labelled polypeptide products were analysed by SDS/PAGE (10 % gel). These were exposed to Fuji RX film or analysed using a Fuji BAS1500 image analyser.

### Generation of fusion polypeptides with glutathione S-transferase (GST) and binding assay *in vitro*

First, the *Sma*I–*Bgl*II fragment of pPC86-BBPori was subcloned into the *Sma*I–*Bam*HI site of pGBT9 (pGBT9-BBPori). Secondly, the *Nco*I–*Pst*I fragment of pGEMTeasyBBP was cloned into the *Nco*I–*Pst*I site of pGBT9-BBPori (pGBT9 BBPfull). Thirdly, the *Eco*RI–*Pst*I fragment of pGBT9-BBPfull was subcloned into the *Eco*RI–*Pst*I site of the pBS-KS vector (pBS-KS-BBPfull). Finally, the *Eco*RI–*Not*I fragment of pBS-KS-BBPfull was subcloned into the *Eco*RI–*Not*I site of the pGEX4T-2 vector (Pharmacia). The procedures for the production and purification of fusion polypeptides were in accordance with the suggestions of the manufacturer. GST protein or fusion derivatives were immobilized on glutathione–agarose beads and incubated with *in vitro*-translated $^{35}$S-labelled proteins for 2 h at 4 °C in a binding buffer [150 mM NaCl, 10 mM Tris (pH 8.0), 0.3 % Nonidet P-40, 1 mM dithiothreitol, 0.5 mM PMSF, 0.25 % BSA] followed by three washes with binding buffer and a final wash with the same buffer without BSA. $^{35}$S-labelled polypeptide products were analysed by SDS/PAGE (10 % gel). These were exposed to Fuji RX film or analysed using a BAS1500 image analyser.

### RNA isolation and Northern-blot analysis

Total RNA was extracted from mouse tissues and cell lines as mentioned previously [8]. About 20 $\mu$g of each total RNA preparation was electrophoresed on 1 % agarose gels containing 1.1 M formaldehyde. The RNA was transferred to Genescreen Plus membrane (NEN Life Science). mRNA was detected with a $^{32}$P-labelled *Eco*RI fragment of pPC86-BBPori (random prime labelling kit, Takara, Otsu, Japan) after hybridization for 18 h at 42 °C in $5 \times$ SSPE [$1 \times$ SSPE is composed of 0.18 M NaCl, 10 mM sodium phosphate (pH 7.7) and 1 mM EDTA], $5 \times$ Denhart's solution ($1 \times$ Denhart's solution is composed of 0.02 % BSA, 0.02 % Ficoll and polyvinyl pyrollidone), 50 % formamide, 0.1 % SDS, 50 $\mu$g/ml heat-denatured salmon testis DNA and radioactive probe. Membranes were washed twice for 15 min each at 65 °C in a solution containing $2 \times$ SSC ($1 \times$ SSC is composed of 0.15 M NaCl and 15 mM sodium citrate) and 0.1 % SDS, then once in $1 \times$ SSC with 0.1 % SDS for 30 min at 65 °C, and finally twice for 15 min each in 0.1 % SSC with 0.1 % SDS at room temperature. The membranes were then autoradiographed at −80 °C using Fuji RX film or analysed using a BAS1500 image analyser. Human 28 S rRNA oligonucleotide (Clontech) was used as an internal control.

### Gel-retardation assay

The recombinant protein (either product of transcription and translation *in vitro* or GST-fusion polypeptides) was incubated with 5 fmol of $^{32}$P-end-labelled double-stranded oligonucleotide in a volume of 10 $\mu$l. Incubation was carried out at room temperature for 20 min. All binding reactions contained 10 mM Tris/HCl (pH 7.5), 4 % glycerol, 50 mM NaC1, 0.5 mM EDTA, 0.5 mM dithiothreitol, 1 mM $MgCl_2$ and 0.5 $\mu$g of poly(dA-dT). Following electrophoresis in a 5 % polyacrylamide Tris/borate/EDTA gel, the gel was dried and subjected to autoradiography at room temperature.

### Transfection experiments

The *Eco*RI–*Sac*II fragment of pPC86-BBPori and *Sac*II–*Not*I fragment of pGEMTeasy-BBPfull were subcloned simultaneously into the *Eco*RI–*Not*I site of the pcDNA3.1HISA plasmid (Invitrogen). Transfection of cells was performed in accordance with the calcium phosphate method [8] with the pGL3 basic vector (Promega) containing the −350 to +54 bp wild-type fragment or the deletion mutant between −170 and −130 [6] of the mouse *pro*$\alpha$2(I) type-I collagen gene with the thymidine kinase promoter/luciferase vector (Promega) as the internal control. Cells were harvested after 48 h and assayed for luciferase activity using the Promega kit.

© 2000 Biochemical Society

## RESULTS

### Cloning of cDNAs for polypeptides that interact with BFCOL1

We used the N-terminal half of the BFCOL1 gene, containing the zinc-finger DNA-binding domain, to clone cDNAs for proteins interacting with BFCOL1 using the yeast two-hybrid system. This domain was chosen mainly on the basis of previous studies that indicated that the C-terminal portion of BFCOL1 interacts with TAF110 (results not shown). We aimed to clarify which molecule interacts with the N-terminal domain of BFCOL1. To begin the identification of some of the proteins that interact with this domain, we used a polypeptide from this DNA as a bait in the yeast two-hybrid system and screened a mouse embryonic fibroblast cDNA library primed with a random hexamer oligonucleotide [8]. In the yeast strain that was used for selection, the plasmid pRS305HISpAS-BFCOL1N was integrated into the genome. In this plasmid, the *Sma*I–*Nsi*I fragment of BFCOL1 was fused to the GAL4-binding domain. By screening 3 million independent colonies from the library, the initial 20 histidine-positive colonies were selected. Among them, one cDNA plasmid yielded a positive result upon retransformation of the parental strain. This cDNA could specifically activate the *his3* gene and $\beta$-galactosidase gene of the yeast strain containing the pRS305HISpAS-BFCOL1N plasmid without activating the *his3* or $\beta$-galactosidase genes of the strain with the pRS305HISpAS control plasmid. This cDNA clone was designated BBP (see below), and was about 850 bp. Partial sequence analysis showed that the cDNA selected by us had no stop codon or poly(A)$^+$ tail. We then attempted to clone the full-length cDNA of BBP by the RACE method. We obtained an approx. 3.2-kb cDNA that extended the approx. 2.3-kb cDNA at the 3′ end; however, we could not obtain further upstream cDNA sequences from the 5′ end. The open reading frame starting from the first methionine codon in the cDNA was 1176 nucleotides in length and encoded a putative polypeptide of 392 amino acids. BBP full-length cDNA had approx. 1.9 kb of a 3′-untranslated sequence. In the GenBank database, the open reading frame sequence of BBP was identical to the murine PTRF cDNA, which induces dissociation of paused ternary transcription complexes [11].

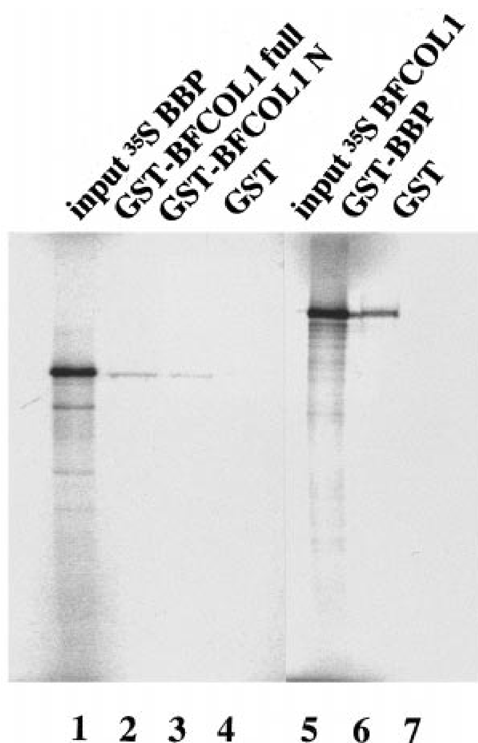


**Figure 1 Binding assay *in vitro***

$^{35}$S-labelled BBP was incubated with GST-BFCOL1 full-length (lane 2), GST-BFCOL1-N (lane 3) or GST alone (lane 4) *in vitro*, washed extensively and electrophoresed on a 10% polyacrylamide SDS gel. Reverse reactions are also shown (lanes 6 and 7).

### Transcription and translation analysis *in vitro*

To confirm that the cDNA was translated precisely, we performed transcription and translation analysis *in vitro*. We could detect the 47-kDa band from the original BBP cDNA and the 58-kDa band from the full-length BBP cDNA, suggesting that BBP full-length cDNA had a fairly long untranslated cDNA sequence (results not shown).

### Binding experiments *in vitro*

To confirm that the BBP could bind to BFCOL1 *in vitro* as well as under the conditions of the yeast two-hybrid system *in vivo*, we carried out a binding analysis *in vitro*. $^{35}$S-Labelled original BBP protein was incubated with GST-BFCOL1 full-length or GST-BFCOL1N recombinant protein [8] and washed extensively and analysed by SDS/PAGE. As shown in Figure 1, a 47-kDa [$^{35}$S]methionine-labelled protein, which bound to GST-BFCOL1 full-length (Figure 1, lane 2) or GST-BFCOL1N (Figure 1, lane 3) was detected, but no proteins that bound to GST alone were detected (Figure 1, lane 4). The reverse experiment using $^{35}$S-labelled BFCOL1 and GST-BBP was also performed, and we obtained the same results (Figure 1, lanes 6 and 7). These results indicate that the polypeptide from the BBP cDNA could bind to the BFCOL1 protein *in vitro* as well as *in vivo* under the conditions of the yeast two-hybrid system.

### Northern-blot analysis

To determine the expression pattern of BBP, a Northern hybridization experiment was performed. Only one 3.2-kb RNA transcript was identified in all the tissues examined (Figure 2A). The expression was fairly strong in the lung, heart and colon (Figure 2A, lanes 3, 4 and 8 respectively). The expression was moderate in the thymus, spleen, kidney and testis (Figure 2A, lanes 2, 6, 7 and 9 respectively). The expression was weak in the brain and liver (Figure 2A, lanes 1 and 5 respectively). This expression pattern is similar to the expression of the type-I collagen gene. In addition, we carried out the same analysis on cell lines (Figure 2B). BBP transcripts were identified strongly in only NIH3T3 fibroblasts (Figure 2B, lanes 8 and 11) and were expressed weakly in the MG63 bone cell line (Figure 2B, lane 10) and very faintly in the UMR106 osteogenic sarcoma cell line (Figure 2B, lane 5). In other cell lines, we could not detect any bands, even after prolonged exposure. These results suggest that BBP is expressed preferentially in cell lines where type-I collagen is expressed.

### DNA-binding experiments

Gel-shift experiments were performed with the $-136$ to $-180$ DNA segment of the mouse pro$\alpha$2(I) collagen promoter to clarify the effect of BBP on BFCOL1 binding to the proximal type-I collagen promoter. As shown in Figure 3, the addition of BBP enhanced the binding of BFCOL1 to the $-136$ to $-180$ fragment (Figure 3, compare lanes 1 and 2). BBP itself did not

© 2000 Biochemical Society

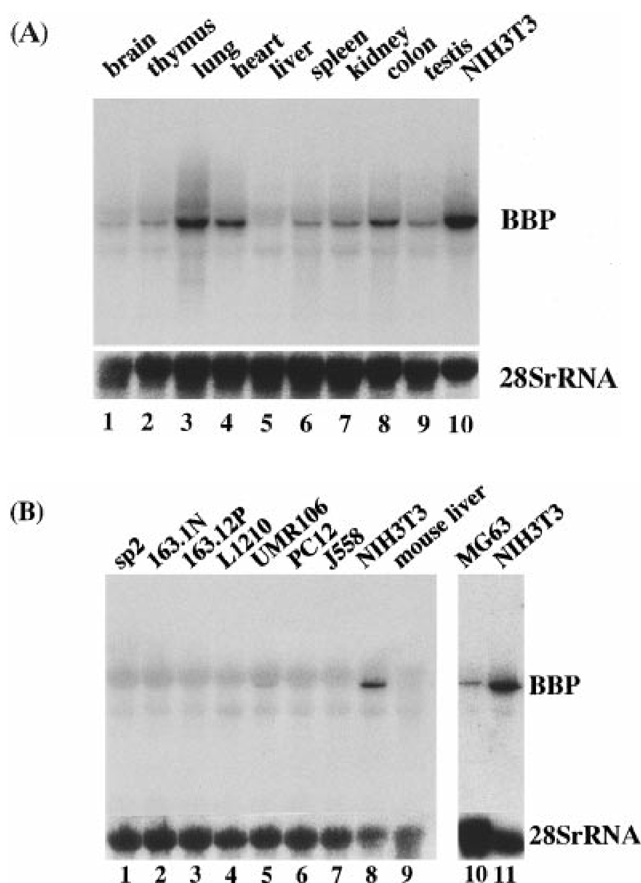


**Figure 2 Northern-blot analysis**

Total RNA was extracted from C57BL6 mouse tissues (**A**) and several cell lines (**B**), electrophoresed, blotted and hybridized with a $^{32}$P-labelled BBP cDNA probe. sp2, 163.1N and 163.12P, hybridoma; L1210, T-cell lymphoma; UMR106, osteogenic sarcoma cell line; PC12, pheochromocytoma; J558, myeloma; MG63, bone cell line. Human 28S rRNA probe was used as an internal RNA-loading control.

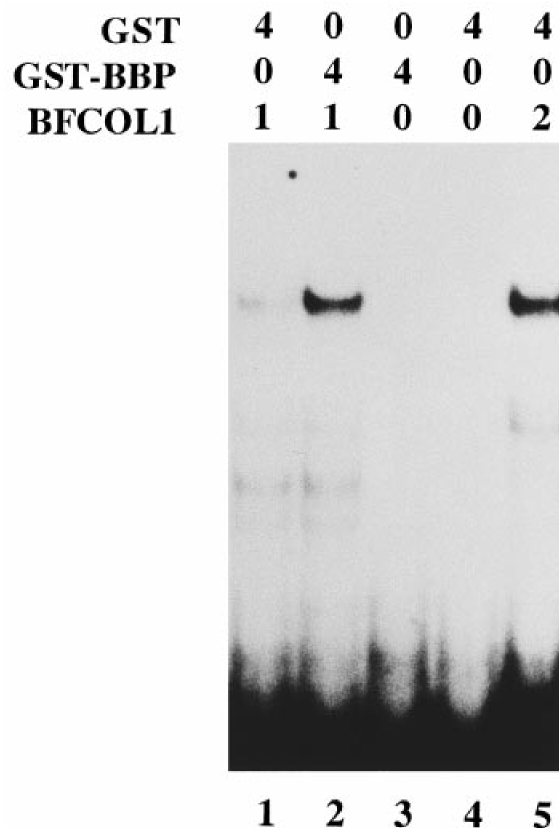


**Figure 3 Gel-shift assays**

Double-stranded $^{32}$P-labelled oligonucleotides containing the sequences between −180 and −136 of the mouse pro$\alpha$2(I) collagen promoter were incubated with the combination of the polypeptide products of full-length BFCOL1 obtained by transcription and translation *in vitro*, GST-fusion BBP products and GST alone. The volumes ($\mu$l) used for the reaction are indicated at the top. Reactions were processed as indicated in the Experimental section.

bind to the promoter (Figure 3, lane 3). This result suggests that BBP might form a complex with BFCOL1 in its zinc-finger domain and influence the binding capacity to the proximal promoter of the mouse type-I collagen gene.

### Functional analysis

To test whether BBP could either activate or inhibit transcription, BBP cDNA was subcloned into a mammalian expression vector, pcDNAHIS, and this DNA was co-transfected with the pro$\alpha$2(I) collagen promoter (−350 to +54) linked to the luciferase reporter gene in NIH3T3 fibroblasts. No activation occurred with the transfection of the BBP expression vector. Otherwise, the BBP expression vector inhibited the promoter activity to about 65 % (Table 1). To test if this suppression was BFCOL1-binding-site-specific, we transfected the BBP expression vector with the deletion mutant between −170 to −130 of the pro$\alpha$2(I) collagen promoter at which BFCOL1 binds [8]. The suppression was detected even in this mutant (about 45 %). However, the suppression was not as strong as that detected in the wild type. These results suggest that BBP had a suppressive effect on the pro$\alpha$2(I) collagen promoter and one suppressive mechanism might be through the involvement of BFCOL1-binding elements.

**Table 1 Effect of BBP on the activity of the mouse pro$\alpha$2(I) collagen promoter**

Transient-expression experiments were performed after DNA transfection into NIH3T3 fibroblasts. Firefly luciferase activities were compared with the activity of the wild-type collagen promoter without the BBP expression vector. In each experiment, the firefly luciferase activities were normalized for transfection efficiency by measuring sea pansy luciferase activities driven by the thymidine kinase promoter. Each construct combination was assayed in duplicate transfections in at least two separate experiments. Statistical analysis showed $P < 0.001$ between constructs (1) and (2), $P < 0.01$ between constructs (3) and (4), and $P < 0.05$ between constructs (1) and (3).

| Construct | BBP | Luciferase activity (%) relative to (1) |
|---|---|---|
| 1. −350 to +54 bp | − | 100.0 ± 19.5 |
| 2. −350 to +54 bp | + | 35.3 ± 13.2 |
| 3. −350 to +54 bp (−170 to −130 deleted) | − | 75.7 ± 19.6 |
| 4. −350 to +54 bp (−170 to −130 deleted) | + | 42.0 ± 19.3 |

## DISCUSSION

We have cloned the cDNAs that interact with the BFCOL1 zinc-finger transcription factor, which binds to the two promoters of the mouse *pro$\alpha$1*(I) and *pro$\alpha$2*(I) collagen genes [8] using the

© 2000 Biochemical Society

yeast two-hybrid system. We designated this cDNA BBP (BFCOL1-binding protein). The full-length cDNA of BBP contains an open reading frame for a polypeptide of 392 amino acids. In the GenBank database, the sequence of the BBP open reading frame is exactly the same as PTRF [11]; however, our cDNA has a fairly long 3′-untranslated region. PTRF interacts with TTF1 (transcription-termination factor 1) [14], which binds to the '*Sal*I box' that is repeated several times downstream of the 3′ end of the pre-rRNA coding region, RNA polymerase I and transcripts containing the 3′ end of pre-rRNA. Hence PTRF has been shown to mediate transcription termination. PTRF cDNA was cloned by screening a human lung fibroblast WI-38 cDNA library and the full-length cDNA was obtained from NIH3T3 cell lines. However, there was no information about the tissue distribution or the function of PTRF in the RNA polymerase II system. We showed that the expression pattern of PTRF is very similar to that of type-I collagen (Figure 2A). Also, its expression was completely limited to the fibroblast and bone cell lines; it was not detected in the blood or neuronal cell lines examined (Figure 2B). We cannot rule out the possibility that PTRF is expressed in the cell lines other than fibroblast-lineage cell lines in very small amounts that we cannot detect by Northern-blot analysis. However, judging from its tissue distribution, one of the functions of PTRF may be related to tissue-specific gene expression, in addition to rRNA transcription, which is ubiquitous. In this article, we have shown that BBP is involved in transcription. First, we showed that PTRF interacts with the DNA-binding domain of BFCOL1 not only in the yeast two-hybrid system but also in the binding reaction *in vitro*. Secondly, although PTRF does not have a DNA-binding motif itself, it could induce BFCOL1 protein to bind more efficiently to the GC-rich sequence of the mouse type-I collagen proximal promoter (Figure 3). In addition, the transient-transfection experiments showed that PTRF decreases the type-I collagen promoter activity (Table 1), either through the BFCOL1-binding site or from other unknown factors. The former possibility is that PTRF enhances the binding of BFCOL1 to the proximal promoter and exerts its inhibitory effect indirectly through BFCOL1, which binds to the type-I collagen promoter. However, we can also speculate on the other possibility that PTRF can affect the interaction of the promoter activity with other transcription factors, especially proteins that have a zinc-finger motif, not only because PTRF has a suppressive effect even on promoters that do not have a BFCOL1-binding site but also because the zinc-finger domain of BFCOL1 interacts with BBP. However, we do not have any evidence to prove the latter possibility at this moment.

So far among the type-I collagen promoters, only positively effective transcription factors have been studied, such as CBF, CCAAT-box binding protein, NF1 (nuclear factor 1) [15], Sp1 and cKrox [16,17]. As inhibitory factors, IF-1 and IF-2 have been speculated upon, but the molecules have not been identified. BFCOL1 might be a candidate for IF-1. Deletion mutants that include the BFCOL1-binding site decrease promoter activity but when mutations are introduced in the BFCOL1-binding site, promoter activity is increased. The C-terminal domain of BFCOL1 that is not the PTRF-binding portion has a transcription activator, as shown in the previous study [8]. This may suggest that a stronger transcription activator than BFCOL1 binds to the BFCOL1-binding site competitively. If one regulatory mechanism of the promoter activity is competition for the binding of transcription factors, the presence of a modulator of transcription factors such as PTRF that changes the affinity for BFCOL1 binding is important. Therefore it is reasonable that PTRF is expressed only in the fibroblast-lineage cell lines that express type-I collagen. Merchant et al. reported that ZBP-89, a rat homologue of BFCOL1, inhibits induction by the epidermal growth factor of the gastrin promoter [18]. In addition, the ht$\beta$ human homologue of BFCOL1 acts as an activator of the T-cell receptor promoter [19]. BFCOL1 might act as a suppressor of type-I collagen promoters. The molecular mechanisms underlying these differences are unknown. However, our finding that PTRF, a cofactor of BFCOL1, is not expressed in either human stomach cell lines (results not shown) or T-cell lymphoma lines suggests that ZBP-89 and ht$\beta$, with or without the cofactors that interact with them, such as PTRF, might influence the function of each target promoter.

In brief, PTRF, a BFCOL1-binding protein, appears to be expressed in a tissue-specific manner and has influence on the proximal region of the pro$\alpha$2(I) collagen promoter and probably controls the activity of this promoter in conjunction with other ubiquitous DNA-binding proteins, as well as tissue-specific transcription factors. The cause of fibrotic diseases, such as lung fibrosis, cirrhosis and scleroderma, are unknown. Moreover, treatment and prevention are difficult. PTRF and BFCOL1 may be inhibitory to the expression of type-I collagen genes and could be future targets for the treatment of these diseases.

We thank Dr S. Saga for providing the cell lines. This work was supported by the fund for comprehensive research on aging and health.

## REFERENCES

1 Vuorio, E. and de Crombrugghe, B. (1990) Annu. Rev. Biochem. **59**, 837–872
2 Schmidt, A., Rossi, P. and de Crombrugghe, B. (1986) Mol. Cell. Biol. **6**, 347–354
3 Hatamochi, A., Paterson, B. and de Crombrugghe, B. (1986) J. Biol. Chem. **261**, 11310–11314
4 Hatamochi, A., Golumbek, P. T., Van Schaftingen, E. and de Crombrugghe, B. (1988) J. Biol. Chem. **263**, 5940–5947
5 Maity, S. N., Golumbek, P. T., Karsenty, G. and de Crombrugghe, B. (1988) Science **241**, 582–585
6 Hasegawa, T., Zhou, X., Garrett, L. A., Ruteshouser, E. C., Maity, S. N. and de Crombrugghe, B. (1996) Nucleic Acids Res. **24**, 3253–3260
7 Bou-Gharios, G., Garrett, L. A., Rossert, J., Niederreither, K., Eberspaecher, H., Smith, C., Black, C. and de Crombrugghe, B. (1996) J. Cell Biol. **134**, 1333–1344
8 Hasegawa, T., Takeuchi, A., Miyaishi, O., Isobe, K. and de Crombrugghe, B. (1997) J. Biol. Chem. **272**, 4915–4923
9 Karsenty, G. and de Crombrugghe, B. (1990) J. Biol. Chem. **265**, 9934–9942
10 Karsenty, G. and de Crombrugghe, B. (1991) Biochem. Biophys. Res. Commun. **177**, 538–544
11 Jansa, P., Mason, S. W., Hoffmann-Rohrer, U. and Grummt, I. (1998) EMBO J. **17**, 2855–2864
12 Sikorski, R. S. and Hieter, P. (1989) Genetics **122**, 19–27
13 Gietz, D., St. Jean, A., Woods, R. A. and Schiestl, R. H. (1992) Nucleic Acids Res. **20**, 1425
14 Bartsch, I., Schoneberg, C. and Grummt, I. (1988) Mol. Cell. Biol. **8**, 3891–3897
15 Oikarinen, J., Hatamochi, A. and de Crombrugghe, B. (1987) J. Biol. Chem. **262**, 11064–11070
16 Gálera, P., Musso, M., Ducy, P. and Karsenty, G. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 9372–9376
17 Gálera, P., Park, R.-W., Ducy, P., Mattei, M.-G. and Karsenty, G. (1996) J. Biol. Chem. **271**, 21331–21339
18 Merchant, J. L., Iyer, G. R., Taylor, B. R., Kitchen, J. R., Mortensen, E. R., Wang, Z., Flintoft, R. J., Michel, J. B. and Bassel-Duby, R. (1996) Mol. Cell. Biol. **16**, 6644–6653
19 Wang, Y., Kobori, J. A. and Hood, L. (1993) Mol. Cell. Biol. **13**, 5691–5701

Received 2 September 1999/16 November 1999; accepted 4 January 2000

© 2000 Biochemical Society