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The Potential for Bidirectional Promoter Activity of the Human PDGF-A Chain Gene

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ABSTRACT

Platelet-derived growth factor (PDGF) is a heterodimeric glycoprotein consisting of A and B chains. A functional promoter had been identified in the 5' flanking region of the human PDGF-A chain gene. We found that the PDGF-A chain promoter region possesses the potential for bidirectional activity. This bidirectional promoter activity is influenced by the 5'-untranslated region (5'-UTR) and serum concentration. The 5'-UTR may regulate expression of the PDGF-A chain by transcription in the opposite direction.

Key words: *PDGF-A, Bidirectional promoter*

INTRODUCTION

Platelet-derived growth factor (PDGF) is a heterodimeric glycoprotein composed of A and B chains, and is one of the major mitogens of cells derived from mesodermal tissue[7)]. The PDGF-A chain is expressed in many tumor cells[4)], and abnormal expression of this gene is considered to be closely related to the process of carcinogenesis. The mechanism of regulation of expression of a PDGF-A chain gene is very complex. The 5' regulatory site has been clarified[25)], but the mechanism of regulation remains to be elucidated. The PDGF-A chain gene possesses the 5'-untranslated region (UTR) of 845 base pair long[25)], and its involvement in the regulation of expression of the gene has been suggested. In a present study, we found that the PDGF-A chain promoter possesses a bidirectional potential whose activity is influenced by the UTR.

MATERIALS AND METHODS

Plasmid construction: Deletion mutants on the 3' side of the 5'-UTR were constructed using various restriction enzymes and exonuclease III. The initiation site on the 5' side was constructed by partial digestion with nuclease S1 and was 25 bp upstream of TATAA for UTR-CAT3, 5'-3', UTR-CAT4, 3'-5' and UTR-CAT5, 3'-5'. Plasmids shown in Fig. 1 were prepared by fusing pSVO-CAT containing only the structural gene of chloramphenicol acetyl transferase (CAT) and fragments of the 5'-UTR. Whether each fragment was correctly constructed was confirmed by sequencing. pSV2CAT included the promoter and enhancer sequences of SV40.

DNA transfection and CAT assay: About 24 hours before transfection, RD cells (a human embryonal rhabdomyosarcoma cell line) were seeded at 5.5 × 10^5 cells per 100 mm Petri dish. Twenty micrograms of each plasmid was transfected by the $CaPO_4$ method. After 3 hours, the cells were treated with 15% (v/v) glycerol in 20 mM Hepes buffer for 3 minutes, washed, and incubated for 48 hours with 0.2%, 10% or 20% FCS. They were then harvested, and 100 μg of lysates was assayed for CAT activity.

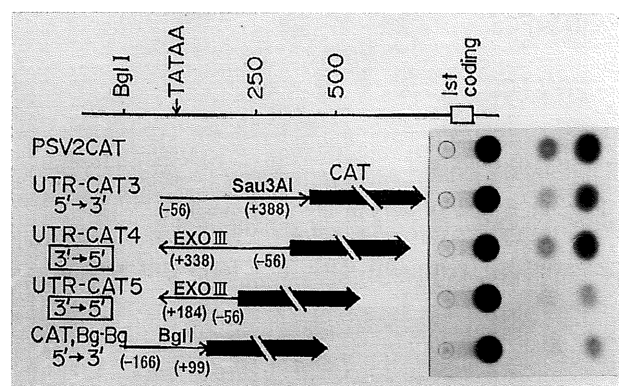

Fig. 1. Plasmid construction and CAT assay UTR-CAT4, 3'-5', and UTR-CAT5, 3'-5', in which the fragment was inserted in the direction opposite to that of the CAT gene, showed CAT activity. The transcription start site corresponds to the +1.

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Northern blot analysis: Total cellular RNA was isolated for Northern blot[17]. The probe for the CAT gene was labeled with α-^{32}P dCTP by random primer labeling.

RESULTS

As shown in Fig. 1, UTR-CAT4, 3'–5', and UTR-CAT5, 3'–5', in which the fragment of 5'-UTR is inserted in the direction opposite to that of the CAT gene, showed CAT activity. CAT activity in UTR-CAT4, 3'–5', possessing a long UTR, was greater than that in UTR-CAT5, 3'–5'. UTR-CAT4, 3'–5', showed a decrease in CAT activity due to stimulation with 20% fetal calf serum, while UTR-CAT5, 3'–5' and UTR-CAT3, 5'–3' were not affected by that concentration of serum (Fig. 2). In the RD cells which were transfected by UTR-CAT4, 3'–5', CAT mRNA was detected as shown in Fig. 3, suggesting that transcription of the CAT gene occurred with the fragment inserted in the direction opposite to that of the CAT gene.

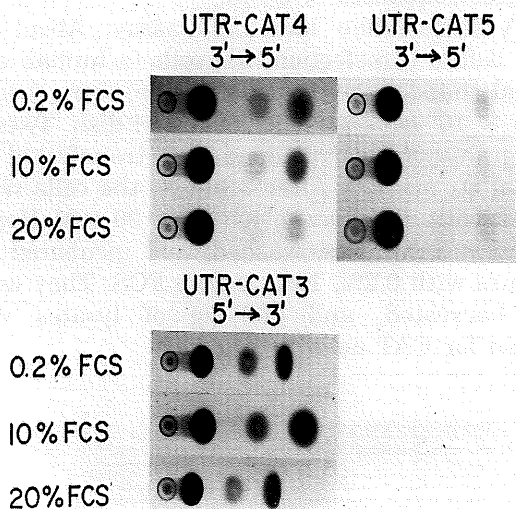

Fig. 2. Effect of serum on CAT activity
The CAT activity of UTR-CAT4, 3'–5', was decreased by treatment with 20% fetal calf serum.

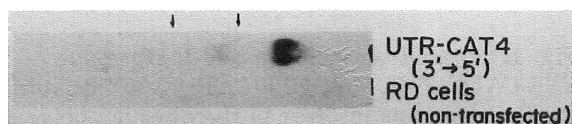

Fig. 3. Northern hybridization
CAT mRNA was observed in a transfectant of UTR-CAT4, 3'–5'.

DISCUSSION

The present study revealed that the PDGF-A chain promoter region possesses the potential for bidirectional activity. There are several reports on bidirectional promoters: SV40[2,9], dihydrofolic reductase gene[21], α1(IV) and α2(IV) collagen genes[5,19], proliferating cell nuclear antigen (PCNA) gene[20], mitochondrial promoters[6], c-Ha-ras gene[16], hypoxanthine phosphoribosyl transferase gene and 3-phosphoglycerate kinase gene[12], and Surf-1 and Surf-2 genes[15]. Although no actual transcript has been demonstrated, the potential for bidirectional promoter activity has been found in the following genes: insulin II gene[8], 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase gene[1], and HTF 9 gene[14].

Among genes with a bidirectional promoter, SV40 early and late transcript and α1 (IV) and α2 (IV) collagen transcript are translated, and they are considered to be divergently transcribed genes. The expression of divergent genes is regulated by a common mechanism, and the products also show functional relationships: e.g., they are involved in a common metabolic pathway, like GAL-1 and GAL-10[13], and associated polypeptides form protein complexes like α1(IV) and α2 (IV) collagen[5,19] and histone H2A and H2B[24].

RNA produced from a bidirectional promoter, whose direction is opposite to that of the structural gene, may also be involved in gene regulation as antisense RNA. In prokaryotes, expression of plasmid Col E1[27], transposon Tn 10[22] and membrane protein Omp C and Omp F genes[18] is regulated by the antisense RNA. In eukaryotes, the antisense RNA may block RNA elongation for the myc gene[3]. In the case of the PDGF-A chain, the promoter region forms an S1-hypersensitive site, showing a single-strand state[28] where an antisense RNA binding may occur. According to Franklin et al[10] paucimolecular PDGF-B chain mRNA may be the antisense RNA of the major c-sis transcript. With regard to the PDGF-A chain gene, however, no antisense RNA against the transcript has been reported.

The majority of divergently transcribed genes are house keeping genes. They are characterized by a defect in the TATAA box. When the TATAA box is deleted from the gene possessing it, divergent transcription occurs[11]. As a second characteristic, the part surrounding the promoter is G-C rich and a GC box is often present. Even if a TATAA box defect is present, divergent transcription does not occur unless the promoter region is G-C rich[23]. The origin of bidirectional transcription is in the methylation-free island[14]. The surrounding part of the PDGF-A chain promoter is very rich in G-C and it possesses GC boxes as previously reported, but a TATAA box is present. The present analysis revealed that bidirectional

promoter activity is influenced by the 5'-UTR. We have previously found that the 5'-UTR regulates the PDGF-A chain gene at the transcriptional and post-transcriptional levels[26]. The present study suggests that the 5'-UTR further regulates expression of the PDGF-A chain by regulating the transcription in the direction opposite to that of the 5'-UTR.

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