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The Human PTFγ/SNAP43 Gene: Structure, Chromosomal Location, and Identification of a VNTR in 5′-UTR[1]

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PTF/SNAPc is a multisubunit complex which specifically recognizes the PSEs of small nuclear RNA genes and activates transcription by RNA polymerase II or III. Here we describe the isolation and characterization of genomic clones encoding the human PTFγ/SNAP43 gene. The gene spans approximately 29 kilobases, and is composed of 9 exons and 8 introns. A major transcription initiation site was identified at the position 58 base pairs upstream of the AUG translation initiator codon on primer extension analysis with HeLa mRNA. The 5′ flanking region lacks a typical TATA box but contains many putative binding sites for various transcription factors, such as Sp1, Oct1, NF1, AP1, E2F, and USF. Immediately downstream of the transcription start site, we found a VNTR of a 17-bp sequence rich in (G+C). Four different alleles with two to five copies of the tandem repeat were identified in 10 individuals examined, indicating a high degree of variation at the PTFγ/SNAP43 locus. In addition, the PTFγ/SNAP43 gene was mapped to human chromosome 14q22 by fluorescence *in situ* hybridization.

Key words: fluorescence *in situ* hybridization, gene organization, PTF/SNAPc, VNTR.

Mammalian snRNA genes contain similar promoter structures, but some (class II) are transcribed by RNA polymerase II whereas others (class III) are transcribed by RNA polymerase III (reviewed in Refs. *1* and *2*). Class II snRNA promoters consist essentially of a PSE, which directs basal transcription, and a DSE, which activates basal transcription. In addition to a PSE and a DSE, class III snRNA promoters contain a TATA box, which functions as the major determinant of RNA polymerase III specificity and directs basal transcription in combination with the PSE (*3, 4*). The PSEs of two classes of snRNA genes are functionally interchangeable and *per se* are not responsible for polymerase selection (*3-5*). However, a transcription factor bound to the PSE may differentially recruit polymerase II or III transcription factors to assist in RNA polymerase selection.

PSE-binding activity has been examined by several laboratories, and referred to as PBP, PTF, or SNAPc (*6-9*). Biochemical analyses showed that PTF/SNAPc is a multisubunit complex of four polypeptides, PTFα/SNAP190, PTFβ/SNAP50, PTFγ/SNAP43, and PTFδ/SNAP45 (*10, 11*). Recently, cDNAs encoding PTF/SNAPc subunits were isolated (*11-16*). Experiments involving antibodies raised against PTF/SNAPc subunits demonstrated that all the PTF subunits are stably associated with each other and essential for both class II and class III snRNA gene transcription. PTFα/SNAP190 functions as the major DNA-binding subunit of the complex and interacts with the transcriptional activator, Oct-1 (*16*). PTFβ/SNAP50 contains potential zinc finger motifs and may be in contact with DNA in the PTF/SNAPc complex (*13, 15*). Both PTFγ/SNAP43 and PTFδ/SNAP45 interact with TBP, which would lead to the formation of RNA polymerase II and III snRNA initiation complexes depending on the promoter context (*11, 12, 14*).

Although the snRNA genes are generally considered to be housekeeping genes essential for cell growth, the expression of some snRNA genes is regulated during the processes of development and differentiation (*17*). In an *in vitro* differentiation system involving mouse F9 embryonic carcinoma cells, for example, the expression of U6 snRNA greatly decreases in the course of differentiation induced with retinoic acid and cyclic AMP (*18*). Importantly, the PSE-binding protein has been reported to be essentially involved in this transcriptional regulation of U6 snRNA (*19*). To provide a basis upon which to examine the regulation of PTF/SNAPc gene expression and to study the coordinate expression of PTF/SNAPc subunits, we have undertaken molecular cloning of human genes for PTF/SNAPc subunits. Here we report the characterization of the human PTFγ/SNAP43 gene.

Screening of a human genomic P1 artificial chromosome (PAC) library (*20*) with the PTFγ/SNAP43 cDNA probe yielded a PAC clone, 294f2, which contains an insert of approximately 100 kb. Southern blot analyses with various PTFγ/SNAP43 cDNA probes indicated that clone 294f2 contains the entire PTFγ/SNAP43 gene. DNA from

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Abbreviations: snRNA, small nuclear RNA; PSE, proximal sequence element; DSE, distal sequence element; PTF, PSE-binding transcription factor; SNAPc, snRNA activating complex; VNTR, variable number of tandem repeats.

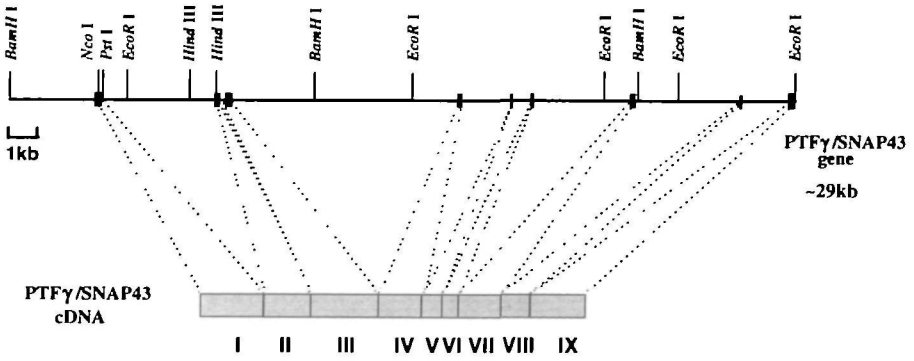

Fig. 1. **Structure of the human PTF γ/SNAP43 gene.** Shown at the top is a restriction map of a PAC clone that contains the whole PTFγ/SNAP43 gene. The exons are represented as solid boxes. At the bottom, the exon numbers and their positions in the PTFγ/SNAP43 cDNA are indicated.

TABLE I. **Exon/intron boundaries of the PTFγ/SNAP45 gene.**

No.	Exon size (bp)	5′splice donor	3′splice acceptor	Intron size (kb)
I	237	TTCTG**GT**GGG.....	.....AATAG**TG**GCA[a]	4.0
II	160	AAAAG**GT**AAT.....	.....TGCAG**AT**CAG	0.16
III	246	TAGAG**GT**AAA.....	.....TTTAG**GA**AAT	8.0
IV	159	GAAAG**GT**ATG.....	.....TTTAG**AA**TTC	2.0
V	69	CGGAG**GT**CAG.....	.....TCCAG**AG**ATG	0.6
VI	63	TACAG**GT**AAG.....	.....TTAAG**GC**ATC	3.6
VII	151	CAAAG**GT**AAC.....	.....TCTAG**GC**AAT	3.7
VIII	96	AACAG**GT**AGA.....	.....TTTAG**AG**TTC	2.1
IX	218			

[a]The GT and AG boundary sequences at the ends of the introns are in boldface letters. The exon sequences are underlined.

clone 294f2 was digested with either *Eco*RI or *Bam*HI, and the fragments that hybridized to PTFγ/SNAP43 cDNA probes were subcloned into the pBluescript vector (*21*). The resulting plasmid DNA was sequenced using primers derived from the PTFγ/SNAP43 cDNA (*22*). The exon/intron boundaries were determined by comparing the sequences to that of the PTFγ/SNAP43 cDNA.

Restriction mapping and Southern hybridization analyses of the overlapping subclones revealed a DNA segment of about 29 kb which contains the PTFγ/SNAP43 gene (Fig. 1). The gene is composed of 9 exons interrupted by 8 introns. The sizes of the exons range from 63 to 246 bp (Table I). Exons 1 and 9 contain the 5′- and 3′-UTR sequences, respectively. The sequences at all the exon/intron junctions conform to the GT/AG rule (*23*). The sizes of introns were determined by either sequencing or PCR amplification using a pair of primers derived from flanking exons. Intron 3 is the largest, spanning about 8 kb. The smallest is intron 2, which is 160 bp.

The transcription initiation site of the PTFγ/SNAP43 gene was determined by primer extension analysis using poly(A)$^+$ RNA from HeLa cells and a primer derived from the first exon of the gene. Two major extension products were obtained, as shown in Fig. 2. A similar result was obtained with another primer derived from a different region of the first exon (data not shown). Since HeLa cells contain PTFγ/SNAP43 alleles with either three or four copies of a 17 bp VNTR in the 5′-UTR (see below), and since the length difference between the two extension products is 17 nt, this result suggested that two products were derived from two mRNA species differing only in the number of 17 bp tandem repeats. Thus, a major transcription start site was assigned to the A residue at the position 58 bp upstream of the AUG translation initiation codon. However, we cannot formally exclude the possibility that only one PTFγ/SNAP43 allele is transcriptionally active

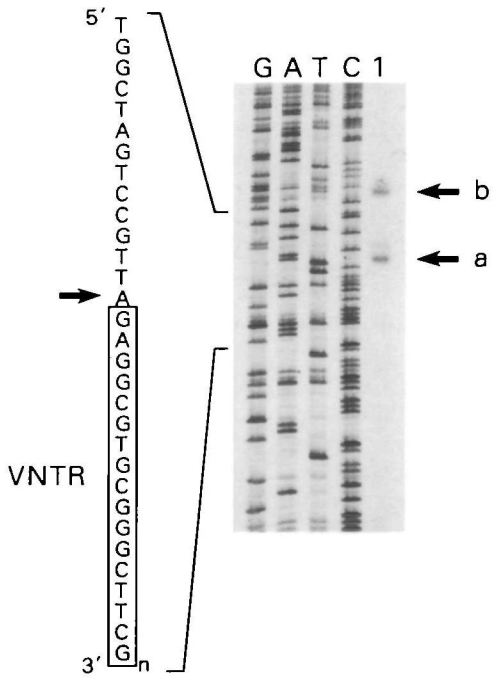

Fig. 2. **Determination of the transcription initiation site by primer extension analysis.** Lane 1, primer extension products with HeLa cell mRNA; lanes G, A, T, and C show DNA sequencing ladders of a HeLa genomic clone with the same primer as used for the primer extension assay. Primer extension analysis was performed with an antisense primer corresponding to nucleotides 178 to 201 shown in Fig. 3. The sequence of the coding strand around the transcription start site is shown on the left. The 17 bp VNTR is boxed. The HeLa genomic clone used in this assay contains three copies of the tandem repeat. Arrow on the left indicates the position assigned to the major 5′ end of the mRNA. Arrows a and b indicate primer extension products presumed to be derived from mRNAs transcribed from HeLa genes containing three and four copies of the tandem repeat, respectively.

in HeLa cells and that its transcription starts at two sites 17 nucleotides apart.

To analyze the promoter region of the PTFγ/SNAP43 gene, we subcloned and sequenced a 3.4 kb *Bam*HI-*Eco*RI fragment containing exon 1 and its 5′-flanking region. Figure 3 shows the sequence of the 5′-flanking region of the PTFγ/SNAP43 gene. Potential regulatory motifs were determined by means of a computer search of the TFD SITES database. No typical TATA box was found at positions −20 to −50 bp upstream of the transcription initiation site. However, the upstream sequence between nucleotides −80 and +1 is relatively GC-rich, 70%, suggesting that it represents the promoter region for the PTFγ/SNAP43 gene. The promoter region contains several DNA elements that bind ubiquitous transcription factors, including Sp1, Oct1, AP1, AP4, C/EBP, E2F, and USF (*24-30*), which may be involved in PTFγ/SNAP43 expression in a variety of cell types. In addition, there are several binding motifs for an erythroid-specific regulator, GATA1 (*31*), suggesting that expression of the PTFγ/SNAP43 gene may be differentially modulated in different tissues. Functional analyses should clarify the roles these binding motifs may play in regulating the expression of the PTFγ/SNAP43 gene.

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-877   GATCAGAAAA GTAAAGTGAG TTGACACTCC ATTTACACAC AGAAAGCCCT
-827   GTTTTCTATT ATATCAGATA CCTGCAACTA AAGAATGATA GCATACCTTT
                  GATA1                  GATA1
-777   CAATCAGAGA ATGATCTAAA GTTTCGAAAA TGTTACTCCA TAGCTAGCTC
-727   CTGGAGGAAG GAAGCTAGGT GGCAGCGTGT ACACAAAAAG GCGATTCATC
-677   CAAATAATAA TATAACAAGA ATATAACAAG TAGGTATTTT CTGCTAAGTG
-627   CTATGTAGCA CAGGGGTGAA TGCGTGGCAG GGCAGGAGGA GGGCTGTTTA
       OCT1
-577   CAAGAGGGTA TGAAATGCAG CTCTTCTTTT CAAGAGTGGT TGCAGAATGC
                  AP4                    C/EBP
-527   CAGGGCTACA AAGAGAGGGC CGCACATTAA GCATCGTATT ATGTGGGCTA
                                        OCT1
-477   CGAGAGTATT AGGGTTCTCG ATGACCAGAA GGAGAATGAG TGCAGTTTGT
                              AP1
-427   TTCCTAGCAA CCTTCTTTAG CTTCTCTTCC CAAGTGGGAG TGGGGTTGGG
-377   GTTGGGACTG TTCTTTCCTA GCTCAAAAGT TCACTTATGT CACACTAGGG
                                        AP1    AP1
-327   TCAGTTTAAA GGAGAGTGCT AGTAAGGGTA AAGTCAAGTT ACATAACTAG
-277   CGGAAAATGA AGACCTAAAA CCGCTCGTGA GCAAACACAA CACTTTAGAC
        ELK1                  USF
-227   GTAAACCAAA GAAGGGTGGA TCTTAGCACG CTGGGTGAAG CAATCTCAGC
                                        C/EBP
-177   CAGTAAAACA TCAGAACGCT TTCACGCACG CGCAGTAAGC ACACGACCGC
                              E2F
-127   TGGCAAGAGC CCGGATAAAG GCGGAGCTAA AGGTGCCGGA TAATGCATCA
        NF1    GATA1                                GATA1
-77    CGGAGGGTGT GCGCACGCCG TAGGGGGCGG GCCGTTCGGG CTTGGTTTCC
                                  SP1
-27    TGAGCGACCA CCGCTGGCTA GTCCGTTAGA GGCGTGCGGG CTTCGGAGGC
                AP4                    +1
+24    GTGCGGGCTT CGGAGGCGTG CGGGCTTCGG AGGCGTGCGG GCTTCGGAGG
+74    CGTGCGGGCT TCGGAGGCGT GCGGGCTTCG GGTGCCATGG GGACTCCTCC
                                        Met
+124   CGGCCTGCAG ACCGACTGCG AGGCGCTGCT CAGCCGCTTC CAGGAGACGG
+174   ACAGTGTACG CTTCGAGGAC TTCACGGAGC TCTGGAGAAA CATGAAGTTC
+224   GGGACTATCT TCTG
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Fig. 3. **Nucleotide sequence of the 5′ region of the human PTFγ/SNAP43 gene.** The sequence shows the entire exon 1 (positive numbers) and 877 bp of the putative 5′-promoter region (negative numbers) of the gene from PAC clone 294f2. The arrow indicates the transcription start site. The potential transcription factor-binding sites are boxed. The 17 bp tandem repeats are underlined and shown in boldface letters. The sequence of the clone derived from HeLa cells is identical except that it contains 3 copies of the 17 bp tandem repeat instead of 6.

Remarkably, as shown in Fig. 3, six copies of a 17 bp tandem repeat were found immediately downstream of the transcription initiation site in PAC clone 294f2 (also see Fig. 4, lane 3). In addition to clone 294f2 derived from human genomic DNA, we sequenced the 5′-flanking region of the PTFγ/SNAP43 clone obtained from HeLa cells by PCR amplification. Three copies of the tandem repeat were found in the 5′-UTR of the gene derived from HeLa cells (Fig. 3 and Fig. 4, lane 2). These results suggested that there was potential polymorphism in the copy number of 17 bp tandem repeats. To examine the presence of a VNTR in the PTFγ/SNAP43 locus, we amplified the region containing tandem repeats by PCR using human DNA samples as templates and analyzed the products by PAGE. As shown in Fig. 4, HeLa cells contain PTFγ/SNAP43 alleles with either 3 or 4 copies of the tandem repeat (lane 1). All human samples examined had at least one allele with two 17 bp repeats (lanes 4 through 13). Five of the 10 samples showed heterozygosity at this locus (lanes 4, 6, and 9-11). In addition to the allele with two repeats, each DNA sample from heterozygotes contained another allele with three (lanes 9 and 11), four (lane 4), or five repeats (lanes 6 and 10), indicating a high degree of variation at the PTFγ/SNAP43 locus. Thus, the VNTR polymorphism in the PTFγ/SNAP43 locus may be used as a valuable genetic marker. The unusual location of the VNTR in the 5′-UTR and its GC-rich sequence may affect expression of the PTFγ/SNAP43 gene by interfering with the translation of its mRNA and thereby make alleles with more repeats be expressed less. Further studies are required to examine this possibility.

To determine the chromosomal location of the human PTFγ/SNAP43 gene for future biomedical studies, we performed chromosome mapping using the fluorescence *in situ* hybridization (FISH) technique (*32*). Metaphase

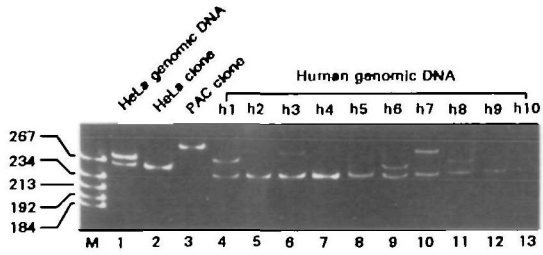

Fig. 4. **Identification of a VNTR in the 5′-UTR of the PTFγ/SNAP43 gene.** Polymerase chain reactions were performed with primers 5′-GATAAAGGCGGAGCTAAAGG-3′ (sense) and 5′-TCCGTGAAGTCCTCGAAGCGTACA-3′ (antisense) so that the amplified PCR products included 17 bp tandem repeats. One nanogram of DNA from genomic clones (lanes 2 and 3), and 50 ng of genomic DNA from HeLa cells (lane 1) or human lymphocytes (lanes 4 to 13) were used as templates. Following initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 20 s, and annealing/extension at 72°C for 45 s were performed. The PCR products were analyzed by electrophoresis on a 10% nondenaturing polyacrylamide gel. The sizes for the marker bands in lane M are listed, in basepairs, at the left.

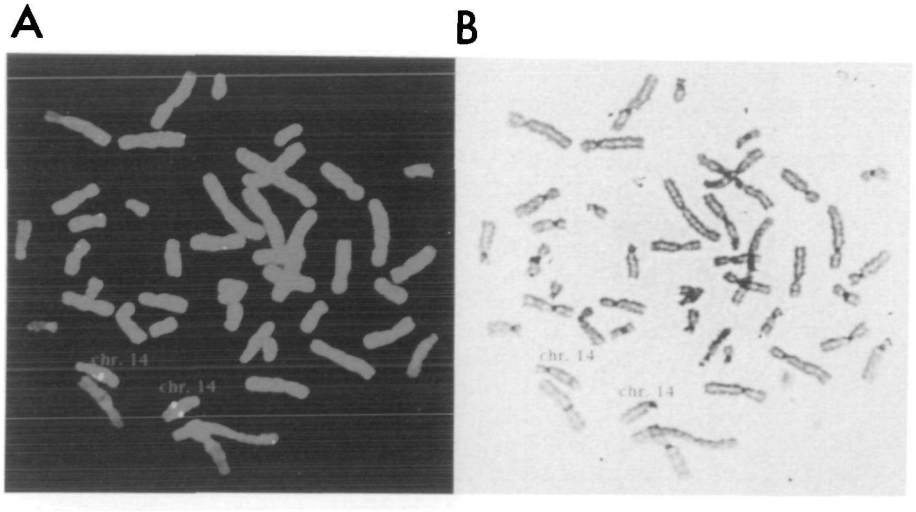

Fig. 5. **Chromosomal localization of the human PTFγ/SNAP43 gene by FISH.** (A) FISH to human metaphase chromosomes with DNA from PAC clone 294f2. PAC DNA was labeled with Biotin-14-dATP and purified on a spin column. Biotinylated probe DNA was dissolved in the hybridization mixture comprising 100 ng of human Cot-1 DNA per μl, 50% formamide, 10% dextran sulfate, and 2× SSC. Probe DNA was denatured at 75°C for 8 min, and then preannealed at 37°C for 30 min. Hybridization was performed at 37°C in a moist chamber for 16 h. The PAC DNA was detected with a fluorescein detection kit (Oncor), and the slides were counterstained with propidium iodide (0.6 μg/ml). (B) G banding pattern on the metaphase spread used for the FISH analysis shown in (A).

chromosomes from normal human lymphocytes were hybridized with a biotinylated DNA probe prepared from the PAC clone, 294f2. Hybridization signals were detected using a fluorescein detection kit (Oncor, Gaithersberg, MD). In the majority of the metaphase spreads examined, specific FISH signals were observed for the long arm of G-banded chromosome 14 (Fig. 5). Of 80 metaphase spreads examined, 78% had two or more fluorescein isothiocyanate signals on four chromatids. No reproducible signal was observed for any other metaphase chromosomes. Thus, the human PTFγ/SNAP43 gene was mapped to chromosomal region 14q22.

In summary, we have characterized the genomic organization of the human PTFγ/SNAP43 gene and have identified a novel VNTR in its 5′-UTR. The molecular cloning of the PTFγ/SNAP43 gene and analysis of the promoter region should facilitate future studies on the regulation of the expression of the PTFγ/SNAP43 gene and the coordinate expression of PTF/SNAPc subunits.

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