Stretches of alternating poly(T-dG), with the capacity to form Z-DNA, are present in human liver transcripts

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A cDNA clone consisting of a stretch of poly(T–dG) alternating residues, a potential Z-DNA forming sequence, was identified in a human cDNA library. The result of Northern blot analysis confirms that this sequence is transcribed into polyadenylated RNA in human liver.

Z-DNA *Human liver RNA* *Northern blot analysis*

1. INTRODUCTION

The existence of a left-handed double-helical conformation of DNA, the so-called Z-DNA, was first observed under particular in vitro conditions [1]. These first studies were done on the sequence poly(dG–dC); other synthetic purine–pyrimidine alternating sequences, such as poly(T–dG)–poly(dC–dA) can also adopt the Z conformation in vitro [2,3]. The left-handed conformation of DNA is probably present in vivo [4,5]. Potential Z-DNA sequences have been identified in the human genome [6]. These findings raise the important question about a possible role of Z-DNA in gene expression. Here, we report the identification of a human cDNA clone from a human endothelial cDNA library, carrying a long stretch of alternating T–dG residues; Northern blot analysis revealed that these sequences are extensively transcribed in human liver.

2. MATERIALS AND METHODS

2.1. *Bacterial strains and phage vectors*

Escherichia coli K12 (strain 71/18) was used for transformation [7]. The M13 derivative mp8 was used as phage vector [8]. Transformation and preparation of double-stranded DNA were done as in [9,10].

2.2. *Enzymes and chemicals*

Avian myeloblastosis virus (AMV) reverse transcriptase was a gift of Dr J. Beard. T4 DNA ligase and restriction endonucleases were gifts of Dr V. Pirrotta. All radioactive compounds were purchased from Amersham Buchler (Braunschweig). Endothelial cell growth factor was purchased from Bethesda Res. Lab. (MD).

2.3. *Purification of mRNA, cDNA synthesis and cDNA shot-gun*

mRNA purification, cDNA synthesis, cDNA shot-gun and Northern Blot Analysis were done as in [11].

2.4. *Endothelial cell culture*

Primary cultures of human endothelial cell (HECC) were obtained from human umbilical cord veins as in [12]. The primary cultures were grown in 199 medium containing 20% FCS, 100 μg ECGF/ml, penicillin G (10 U/ml), streptomycin (1 μg/ml) and fungizone (5 μg/ml).

3. RESULTS AND DISCUSSION

3.1. *Transcription of poly(T–dG) sequences*

A cDNA library from human endothelial

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cultured cells was screened with labelled cDNA from different human tissues and cell lines, to identify tissue-specific sequences. 50 clones were identified as cross-hybridizing with human liver cDNA. Clone mpZ1 carried an insert consisting of a 124 bases long stretch of alternating T–dG residues (fig.1). Poly(T–dG) sequences are present in the human genome as a repeated element with an approximate copy-number of 5×10^4 [6]. Alternating TG sequences have also been found in introns of human actin genes [6,13], and of the human fetal γ globin gene [14]; other poly(T–dG) sequences were also found in the intergenic region between δ and β human globin genes [15] and in the 3′-flanking region of a mouse immunoglobulin gene [16]. The average length of these sequences is about 40 basepairs.

All these data suggest that poly(T–dG) sequences might be transcribed: our data provide direct evidence for their transcription. The identification of such sequence in our cDNA library, using cDNA probes, strongly suggests that this sequence is present in some polyadenylated RNA. This was confirmed by a Northern blot analysis of human liver poly(A^+) RNA using ^{32}P-labelled mpZ1 DNA as probe. The results of the hybridization experiment are shown in fig.2.

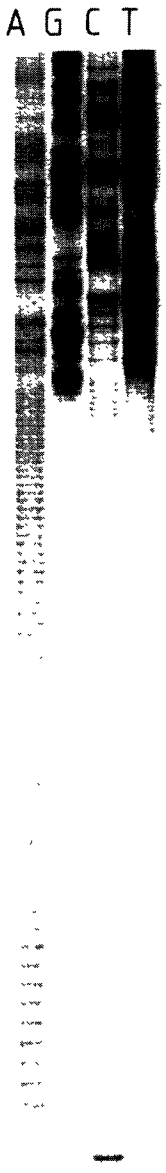

Fig.1. DNA sequence of the clone mpZ1.

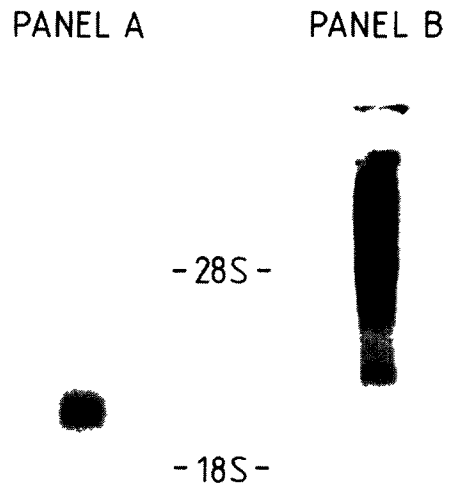

Fig.2. Northern blot analysis: (A) hybridization of a human albumin cDNA clone to total human liver mRNA (30 min exposure); (B) hybridization of mpZ DNA to human liver mRNA (10 days exposure).

In fig.2A we show as control the hybridization of a human albumin cDNA clone to total human liver mRNA. As expected, a single band, corresponding to a 2600-basepair mRNA is detected [11]. In fig.2B the hybridization of the mpZ1 DNA to human liver mRNA is shown. Within a smear in the upper part of the gel, corresponding to RNA molecules longer than 28 S rRNA, it is possible to identify some single bands. One of these comigrates with the 28 S rRNA, the other is smaller (about 2900 basepairs). The presence of a smear in our hybridization indicates that, in human liver, the T–dG sequences are widely transcribed into polyadenylated RNA molecules with relatively long stretches of this sequence. (The high stringency hybridization conditions allow detection of only those hybrids forming more than 50 basepairs.)

3.2. *High instability of the poly(T–dG) sequence*

As for other purine–pyrimidine alternating sequences inserted into plasmid vectors [5], the poly(T–dG) sequence is also unstable. DNA sequence analyses were done after several replicative cycles of the clone mpZ1 in *E. coli* (fig.3).

In fig.3a, the DNA sequence of the clone mpZ1 after several replicative cycles is shown: (⟶) *Eco*RI site of the M13 mp8 polylinker. The phage population, on which DNA sequencing was done, was clearly a mixture of phages carrying inserts of different lengths. In fig.3b the same DNA used for

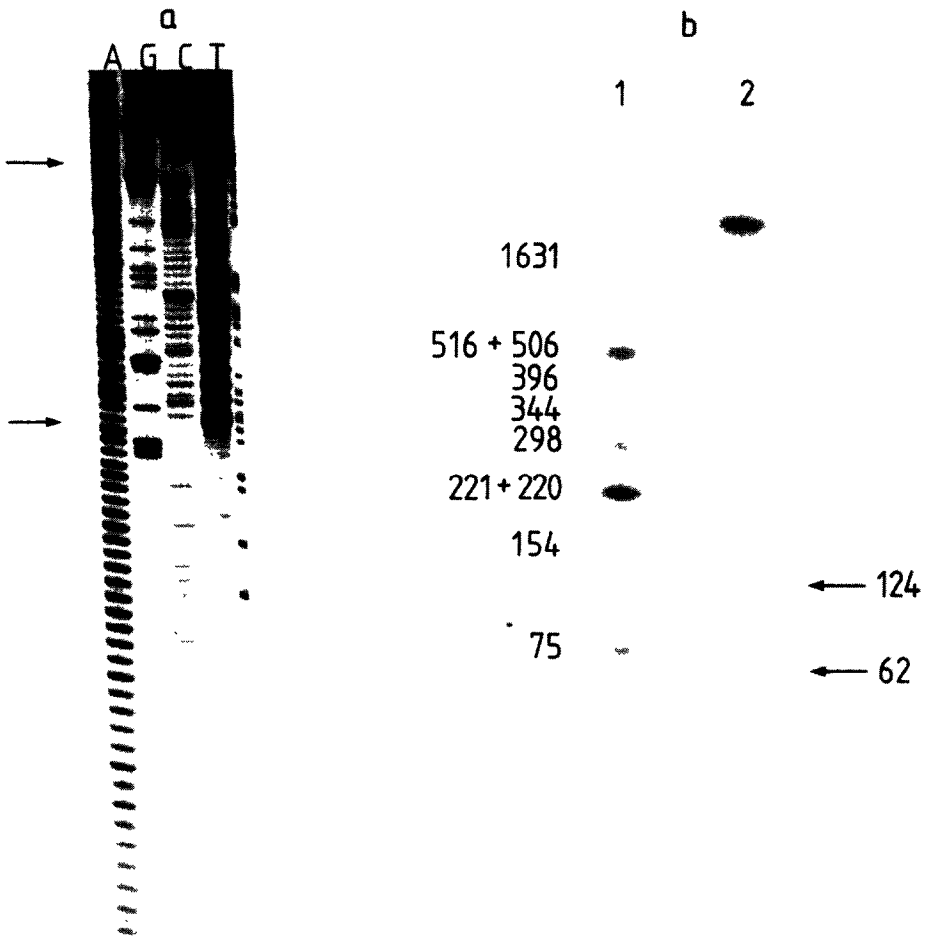

Fig.3. (a) DNA sequence of the clone mpZ1 after several replicative cycles: (⟶) *Eco*RI site of the mp8 polylinker. (b) (1) pBR 322 *Hinf* cut – band lengths are indicated; (2) *Eco*RI–*Bam* H1 digestion of the same DNA sequenced in (A).

the sequence shown in fig.3a has been digested with *Eco*RI and *Bam*H1 to excise the cDNA insert. Two bands are visible: one of 124 basepairs; the other 62 basepairs, clearly the result of a deletion event. We observed this phenomenon in many other cases (not shown). The exceptional instability of the clone mpZ1 is probably due to the particular nature of the inserted sequence.

The discovery of long stretches of alternating purine–pyrimidines in mRNAs raises the question of a role of Z-DNA in gene expression. We are investigating this question and have preliminary evidence that a poly(T–dG) stretch adjacent to an eukaryotic tRNA gene strongly inhibits its transcription (G. Ciliberto, personal communication).

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