

Cloning and analysis of the human S13 ribosomal protein cDNA

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In a hope to isolate genes whose expression was elevated in colon cancer cells, we used a plus/minus screening of a rat colon carcinoma cDNA library. We were thus able to isolate a cDNA clone (B9) encoding the rat S13 ribosomal protein (1). The corresponding mRNA is ~30-fold more abundant in the cancer cells than in normal colon, and ~50-fold more important in lung metastasis than in normal lung. The expression level of this gene was found to be closely correlated with the growth rate of rat cell lines (1).

Unfortunately, the B9 probe was short, and did not hybridize to RNA prepared from human tissues. In order to obtain longer clones encoding the rat S13 ribosomal protein we first rescreened the PROb cDNA library. A longer insert was then used to screen a cDNA library prepared from the T-84 colonic epithelial cell line constructed in the Uni-ZAP XR vector (Stratagene, La Jolla, CA). Inserts were excised with helper phage R408 to generate subclones in the pBluescript plasmid and sequenced by the dideoxy-chain termination method (2) using T7 DNA polymerase. The final sequences were determined from both strands.

This cDNA contains 548 nucleotides and includes a 5' noncoding sequence of 32 nucleotides, an open reading frame of 456 nucleotides, a 3' noncoding sequence of 60 nucleotides, followed by a 19 bp polyA tail (Figure 1). The presumed polyadenylation signal, AATAAA, is located at bases 509–514. The nucleotide sequence in the coding region differs from the rat S13 ribosomal protein sequence (3) in the first position in 6 codons and in the third position in 52 codons. It encodes a sequence of 151 aminoacids which is identical to the rat protein. This highly conserved primary structure of ribosomal proteins across species has been observed for other ribosomal proteins (4, 5).

Northern blot analysis revealed that the size of the human S13 ribosomal protein mRNA is approximately 600 bp. This probe hybridized to 8–10 genomic DNA fragments, probably representing one (or a few) functional S13 gene and a family of nonfunctional pseudogenes, as it has been shown for many other ribosomal protein genes (6, 7).

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hs13          CGCTCTCCTTTTCGTTGCCTGATCGCCGCCATC
rs13          -TC--T-C-T--A--C-T-----
hs13 ATG GGT CGC ATG CAT GCT CCC GGG AAG GGC CTG TCC CAG TCG GCT
rs13 --- --- --- --- ---C --- --- --- --- --- --- --- --- ---G
      Met Gly Arg Met His Ala Pro Gly Lys Gly Leu Ser Gln Ser Ala
hs13 TTA CCC TAT CGA CGC AGC GTC CCC ACT TGG TTG AAG TTG ACA TCT
rs13 C-G --- -C -C -T --- -G --- -G --- C- --- --- ---G ---
      Leu Pro Tyr Arg Arg Ser Val Pro Thr Trp Leu Lys Leu Thr Ser
hs13 GAC GAC GTG AAG GAG CAG ATT TAC AAA CTG GCC AAG AAG GGC CTT
rs13 --- --- --- --- -A -A --- --- -G T- --- --- -A --- -G
      Asp Asp Val Lys Glu Gln Ile Tyr Lys Leu Ala Lys Lys Gly Leu
hs13 ACT CCT TCA CAG ATC GGT GTA ATC CTG AGA GAT TCA CAT GGT GTT
rs13 --- --- -C --- -A --- -G --- -G --- -G --- -C --- ---G ---
      Thr Pro Ser Gln Ile Gly Val Ile Leu Arg Asp Ser His Gly Val
hs13 GCA CAA GTA CGT TTT GTG ACA GGC AAT AAA ATT TTA AGA ATT CTT
rs13 --- -G -C --- --- --- -C -A --- --- -C -G --- ---C ---
      Ala Gln Val Arg Phe Val Thr Gly Asn Lys Ile Leu Arg Ile Leu
hs13 AAG TCT AAG GGA CTT GCT CCT GAT CTT CCT GAA GAT CTC CAT
rs13 --- -C -A -C --- -C --- --- --- -G --- --- --- --- ---
      Lys Ser Lys Gly Leu Ala Pro Asp Leu Pro Glu Asp Leu Tyr His
hs13 TTA ATT AAG AAA GCA GTT GCT GTT CGA AAG CAT CTT GAG AGG AAC
rs13 --G --C --- --- --- --- -C A- --- --- -C --- --- --- ---
      Leu Ile Lys Lys Ala Val Ala Val Arg Lys His Leu Glu Arg Asn
hs13 AGA AAG GAT AAG GAT GCT AAA TTC CGT CTG ATT CTA ATA GAG AGC
rs13 --- -A --- --- --- --- --- --- -C --- --- --- -T --- ---
      Arg Lys Asp Lys Asp Ala Lys Phe Arg Leu Ile Leu Ile Glu Ser
hs13 CGG ATT CAC CGT TTG GCT CGA TAT TAT AAG ACC AAG CGA GTC CTC
rs13 A-A --- --- -G C- --- --- -C --- --- --- -T --- ---G ---
      Arg Ile His Arg Leu Ala Arg Tyr Tyr Lys Thr Lys Arg Val Leu
hs13 CCT CCC AAT TGG AAA TAT GAA TCA TCT ACA GCC TCT GCC CTG GTC
rs13 --A --- -C --- --- --- -G --- -C --- --- --- -T --- ---G
      Pro Pro Asn Trp Lys Tyr Glu Ser Thr Ala Ser Ala Leu Val
hs13 GCA TAAATTGTCTGTACTCAAGCAATAAAAATGATTGTTTAAACGT (A)n
rs13 --- -GCTGT--C---A-----C-C-T-GAGTAAA
      Ala

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Figure 1. Comparison between the cDNA sequences of human and rat S13 ribosomal protein. The top lane indicates the determined sequence of the cDNA coding for the human S13 ribosomal protein. The second lane indicates positions at which the nucleotide sequence of the rat cDNA differs. The third lane indicates the amino-acid sequence. The probable polyadenylation signal is underlined.

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