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Molecular cloning and nucleotide sequences of cDNAs specific for rat liver ribosomal proteins S17 and L30

(Hybridization-translation assay; recombinant plasmid; Maxam–Gilbert technique; automatic Edman degradation)

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SUMMARY

cDNA clones coding for rat liver ribosomal proteins S17 and L30 have been isolated by positive hybridization-translation assay from a cDNA library prepared from 8-9S poly(A)$^+$RNA from free polysomes of regenerating rat liver.

The cDNA clone specific for S17 protein (pRS17-2) has a 466-bp insert with the poly(A) tail. The complete amino acid (aa) sequence of S17 protein was deduced from the nucleotide sequence of the cDNA. S17 protein consists of 134 aa residues with an M_r of 15377. The N-terminal aa sequence of S17 protein determined by automatic Edman degradation is consistent with the sequence data. The aa sequence of S17 shows strong homology (76.9%) to that of yeast ribosomal protein 51 [Teem and Rosbash, Proc. Natl. Acad. Sci. USA 80 (1983) 4403–4407] in the two-thirds N-terminal region.

The cDNA clone specific for L30 protein (pRL30) has a 394-bp insert. The aa sequence of L30 protein was deduced from the nucleotide sequence of the cDNA. The protein consists of 114 aa residues with an M_r of 12652. When compared with the N-terminal aa sequence of rat liver L30 protein [Wool, Annu. Rev. Biochem. 48 (1979) 719–754], pRL30 was found not to contain the initiation codon and 5′-noncoding region.

The cDNA showed twelve silent changes in the coding region, one point mutation and one base deletion in the 3′-noncoding region, compared with mouse genomic DNA for L30 protein [Wiedemann and Perry, Mol. Cell Biol. 4 (1984) 2518–2528].

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Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to mRNA; ds, double stranded; nt, nucleotide(s); PA, polyacrylamide; Pu, purine; ^{R}, resistant; rpe-mRNA, mRNA enriched for ribosomal protein-coding sequences; Tc, tetracycline.

INTRODUCTION

There have been several reports that the syntheses of rRNAs and ribosomal proteins are coordinately regulated in proliferating mammalian cells (for review, see Warner et al., 1980). To elucidate the mechanism of coordinated syntheses of ribosomal

proteins, it would help to determine the sequences of all the ribosomal protein genes. As of now, the N-terminal aa sequences of twelve rat liver ribosomal proteins, and the complete sequence of rat P2 (Lin et al., 1982), L37 (Lin et al., 1983) and L39 proteins (Lin et al., 1984) have been reported. Recently, the complete sequences of mouse L30 (Wiedemann and Perry, 1984), mouse L32 (Dudov and Perry, 1984), rat S26 (Kuwano et al., 1985) and rat S11 (Tanaka et al., 1985) proteins were deduced from the nucleotide sequences of cloned DNAs.

To obtain more information on these problems we constructed cDNA clones specific for rat liver ribosomal proteins S17 and L30, and determined their nucleotide sequences. From these sequences we deduced the aa sequences.

MATERIALS AND METHODS

(a) Preparation of poly(A)$^+$mRNA for ribosomal proteins

mRNA enriched for ribosomal protein-coding sequences (rpe-mRNA) was prepared from poly(A)$^+$RNA from free polysomes of regenerating rat liver by sedimentation through a 5–20% sucrose density gradient (Kuwano et al., 1985).

(b) cDNA synthesis and transformation of *E. coli* χ1776

The ds cDNA was synthesized from rpe-mRNA with AMV reverse transcriptase as described previously (Nabeshima et al., 1982). The methods of Land et al. (1981) were used to prepare longer cDNA specific for S17 (cloned in plasmid pRS17-2). Insertion of cDNA into pBR322 and transformation of *E. coli* χ1776 were performed as described previously (Nabeshima et al., 1982).

(c) Colony hybridization

Transformed colonies were screened by colony hybridization (Grunstein and Hogness, 1975) with [^{32}P]cDNA or the nick-translated insert of [^{32}P]pRS17-1 as a probe, (see RESULTS AND DISCUSSION, section a).

(d) Positive hybridization-translation assay of cDNA

Positive hybridization-translation assay of cDNA was carried out as described by Meyuhas and Perry (1980) with 10 μg of plasmid DNA and 10 μg of rat liver poly(A)$^+$ mRNA for hybridization. Hybridized RNA was assayed in a RNase-treated rabbit reticulocyte lysate supplemented with [^{35}S]methionine (Amersham, U.K.; 800 Ci/mmol). The translation products were then analysed by two-dimensional electrophoresis (Lin et al., 1976) followed by fluorography.

(e) Southern blot hybridization

For measurement of the size of an inserted DNA, plasmid DNA was digested with *Pst*I, subjected to electrophoresis on 1% agarose gel and transferred to a nitrocellulose filter by the procedure of Southern (1975). For use as a hybridization probe, the inserted DNA of recombinant plasmid selected by hybridization-translation assay was nick-translated with *E. coli* polymerase I (Takara Shuzo, Kyoto, Japan) with [α-^{32}P]dCTP (Amersham, 7000 Ci/mmol).

(f) Sequence analyses of cDNA clones

The inserted DNAs of the recombinant plasmids were cleaved with restriction endonucleases (see Fig. 2) (Takara Shuzo), and the 5′-ends were dephosphorylated with bacterial alkaline phosphatase (Takara Shuzo) and labelled with [γ-^{32}P]ATP (Amersham, 5000 Ci/mmol), using T4 polynucleotide kinase (Takara Shuzo). In the case of pRS17-2, the 3′-ends of *Taq*I and *Hin*dIII sites were labelled by the repair reaction with [α-^{32}P]dCTP (Amersham, 7000 Ci/mmol) using *E. coli* DNA polymerase I (Klenow fragment) (Takara Shuzo). In the case of pRL30, the 3′ end of the *Pst*I site was labelled with [α-^{32}P]dideoxy ATP (Amersham, 5000 Ci/mmol) using terminal deoxynucleotidyl transferase. The end-labelled DNA fragments were sequenced by the method of Maxam and Gilbert (1977).

(g) Determination of partial amino acid sequence

Ribosomal proteins prepared from rat liver were separated by two-dimensional PAGE (Ogata and Terao, 1979). S17 was extracted and purified by the method of Dianoux et al. (1981). The partial aa sequence of S17 was determined by automatic Edman degradation in an Applied Biosystems sequenator 470A.

RESULTS AND DISCUSSION

(a) Identification of recombinant plasmids containing cDNAs specific for ribosomal proteins S17 and L30

Of 888 Tc^R colonies, 119 that showed strong positive signals on in situ hybridization with [^{32}P]cDNA were selected. From these 119 colonies, 11 that should contain cDNA specific for particular ribosomal proteins were selected by hybridization-translation assay.

Recombinant plasmids pRS17-1 and pRL30 were shown by hybridization-translation assay to be present in the cDNA clones specific for ribosomal protein S17 (Fig. 1A) and L30 (Fig. 1B), respectively. The inserted DNA from pRS17-1 was, however, too short to cover the entire coding sequence of the S17 protein (not shown). Therefore, 4900 Tc^R colonies were screened by colony hybridization, using pRS17-1 as a probe. Specific cDNA clones were further selected from the positive clones by Southern blot hybridization and the sizes of their inserts were determined. Two clones, pRS17-2 and pRS17-3 were obtained.

(b) N-terminal amino acid sequence of S17

The sequence of 33 aa residues in the N-terminal region of S17 protein was determined (see Fig. 3 and MATERIALS AND METHODS, section g). The aa sequence begins with glycine. The N-terminal aa sequence was found to be identical to that deduced from the nt sequence, as described below.

(c) Nucleotide sequence of S17 cDNA

The nucleotide sequences of the pRS17-2 and pRS17-3 inserts were determined. The restriction maps and the sequencing strategies are shown in Fig. 2. The inserted DNA of pRS17-2 has a coding sequence of 408 bp, a 5′-leader sequence of 24 bp and a 3′-noncoding sequence of 34 bp besides the poly(A) tail (Fig. 3). A putative polyadenylation signal AATAAA is 10 nt upstream of the poly(A) tail. The nucleotide sequence of the pRS17-3 insert is the same as that of pRS17-2 except that pRS17-3 has a partial sequence of pRS17-2 (Figs. 2 and 3).

From the nt sequence of the pRS17-2 insert, we deduced the aa sequence of S17 protein. The sequence of the 33 N-terminal aa residues of S17 protein determined by automatic Edman degradation, coincides perfectly with the deduced sequence. S17 was deduced to consist of 134 aa residues with an M_r of 15377, which is consistent with the reported M_r of 16000 (Kenmochi et al., 1981). The aa composition, calculated from the deduced aa sequence of S17 protein was similar to that of S17 reported by Collatz et al. (1977). These results indicate that pRS17-2 contains the entire coding sequence of S17.

The aa sequence of S17 protein is rich in basic aa; about 20% of the residues are lysine, arginine and histidine, whereas 7% of them are glutamic and aspartic acids. The basic aa residues are concentrated in the N-terminal half of the protein, while the C-terminal half is rich in acidic aa residues. This feature is similar to those of several histones (Isenberg, 1979), yeast L29 ribosomal protein (Käufer et al., 1983) and mouse L32 ribosomal protein (Dudov and Perry, 1984). Assuming that the protein is not modified, its isoelectric point is 10.0, which is reasonable judging from its basic character.

We found strong homology in the aa sequences of S17 protein and yeast ribosomal protein 51 (Teem and Rosbash, 1983), a protein which may be important in cell proliferation (Aborich and Rosbash, 1984). The pattern of this homology is very interesting: the N-terminal portions (residues 1 to 91) of these two proteins have strong homology (76.9%), whereas the C-terminal portions (residues 92 to 134) have no significant homology. The N-terminal halves of these two proteins may be important for their function.

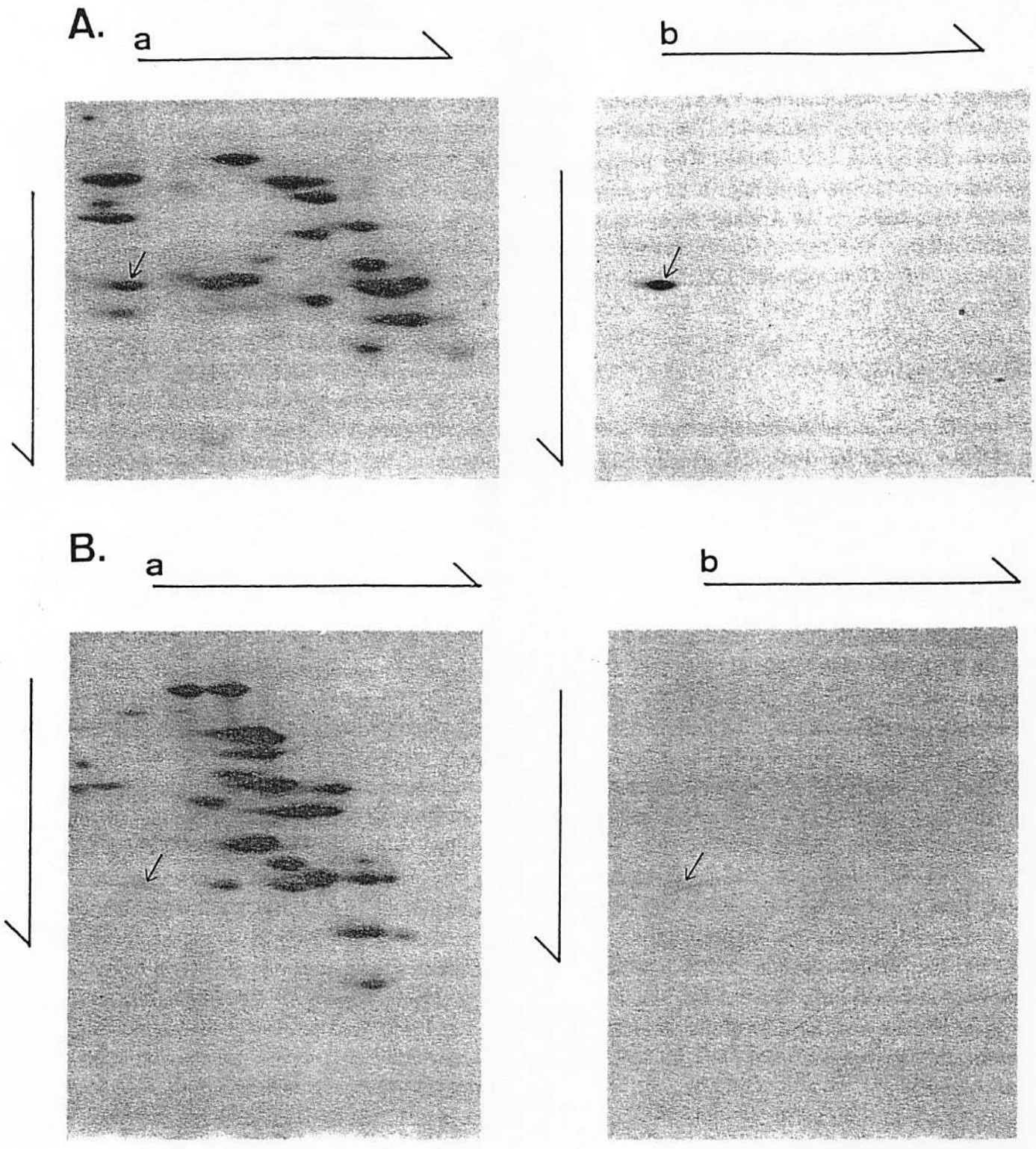

Fig. 1. Two-dimensional PA gel electropherogram of the translation products (see MATERIALS AND METHODS, section **d**). 30 μg of carrier 40S ribosomal proteins were used in (A) and 60 μg of carrier 60S ribosomal proteins were used in (B). The gels were stained with Coomassie brilliant blue (a) followed by fluorography (b). Horizontal and vertical arrows indicate the directions of first- and second-dimension electrophoresis, respectively. Arrows in the gels point to: A, S17; B, L30.

(d) Nucleotide sequence of L30 cDNA

The restriction map and the sequencing strategy are shown in Fig. 2. The nt and deduced aa sequences of the pRL30 insert are shown in Fig. 4. The inserted DNA of pRL30 has a coding sequence of 345 bp and a 3′-noncoding sequence of 49 bp. The N-terminal sequence of 32 aa deduced from the nt sequence of pRL30 coincides perfectly with the N-terminal aa sequence reported by Wool (1979),

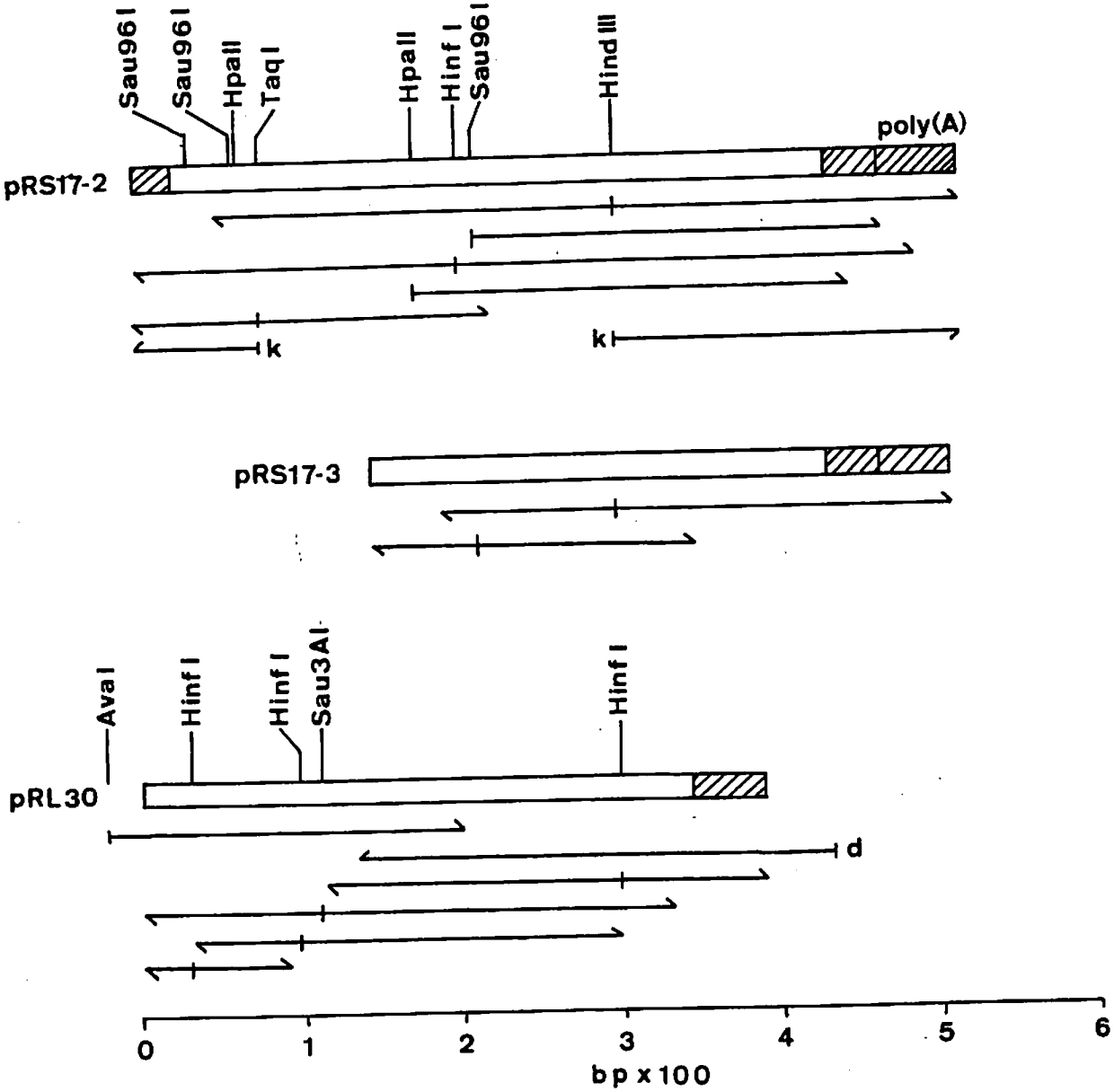

Fig. 2. Sequencing strategy and restriction maps of pRS17-2, pRS17-3 and pRL30 cDNAs. Open bars indicate the coding regions of S17 and L30 mRNAs, and hatched bars indicate noncoding regions. Vertical lines in the 3′-noncoding regions indicate the boundaries of poly(A) tails. Arrows indicate direction (5′ → 3′) and the length of sequences determined with the [γ-^{32}P]ATP label. k indicates 3′-end labelling by DNA polymerase I (Klenow fragment) using [α-^{32}P]dCTP. d indicates 3′-end labelling by terminal deoxynucleotidyl transferase using [α-^{32}P]dideoxy ATP. The scale is shown at the bottom.

indicating that pRL30 is a clone specific for rat L30 protein, although the clone may lack the initiation codon. After we had determined the nt sequence, we learned that Wiedemann and Perry (1984) had obtained genomic clones of mouse L30 protein and determined the nt sequence. Their sequences also showed that pRL30 lacked only the initiation codon. Furthermore, our pRL30 contains the 3′-noncoding region corresponding to the 3′-terminal region of the terminal exon 5 of the mouse L30 gene. The entire nt sequence of cDNA specific for L30 was found to be well conserved in the rat and mouse, and in particular the aa sequences deduced from the nt sequences are the same in the two species. It is noticeable, however,

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                                                                   CCTCTTTTACCGAGACCCGTCAAC
     1                                  10                                    20
    Gly Arg Val Arg Thr Lys Thr Val Lys Lys Ala Ala Arg Val Ile Ile Glu Lys Tyr Tyr Thr Arg
ATG GGC CGC GTT CGC ACC AAG ACT GTG AAG AAG GCG GCC CGG GTC ATC ATC GAG AAG TAC TAC ACG CGC

                            30                                  40
Leu Gly Asn Asp Phe His Thr Asn Lys Arg Val Cys Lys Glu Ile Ala Ile Ile Pro Ser Lys Asn Leu
CTG GGT AAT GAC TTT CAC ACC AAC AAG CGC GTG TGC AAG GAG ATC GCC ATC ATC CCC AGC AAA AAC CTT
                                                                             *
                    50                                  60
Arg Asn Lys Ile Ala Gly Tyr Val Thr His Leu Met Lys Arg Ile Gln Arg Gly Pro Val Arg Gly Ile
CGG AAC AAG ATA GCC GGC TAT GTC ACG CAT CTG ATG AAG CGG ATT CAG AGA GGT CCT GTG AGA GGC ATC

  70                                        80                                    90
Ser Ile Lys Leu Gln Glu Glu Glu Arg Glu Arg Arg Asp Asn Tyr Val Pro Glu Val Ser Ala Leu Asp
TCC ATC AAG TTG CAG GAA GAA GAG AGG GAG AGG AGA GAT AAT TAT GTT CCT GAG GTC TCA GCC CTA GAT

                                100                                 110
Gln Glu Ile Ile Glu Val Asp Pro Asp Thr Lys Glu Met Leu Lys Leu Leu Asp Phe Gly Ser Leu Ser
CAG GAG ATC ATT GAG GTA GAT CCT GAC ACC AAG GAA ATG CTG AAG CTT CTG GAC TTT GGC AGT CTC TCC

                    120                                 130             134
Asn Leu Gln Val Thr Gln Pro Thr Val Gly Met Asn Phe Lys Thr Pro Arg Gly Ala Val
AAC CTC CAG GTC ACT CAG CCT ACG GTT GGG ATG AAT TTC AAA ACA CCA CGT GGA GCT GTT TAA TCCATTA

TGCCATATTTTC AATAAA CCTGAAAAC poly(A)tail
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Fig. 3. Nucleotide sequences and deduced aa sequences of pRS17-2 and pRS17-3 cDNAs. Overlining indicates aa residues determined by peptide sequencing of S17 protein. The asterisk indicates the start point of the pRS17-3 nucleotide sequence.

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  1                                     10                                    20
Val Ala Ala Lys Lys Thr Lys Lys Ser Leu Glu Ser Ile Asn Ser Arg Leu Gln Leu Val Met Lys Ser
GTG GCT GCA AAG AAG ACG AAA AAG TCT CTG GAG TCA ATC AAC TCT AGG CTC CAA CTT GTT ATG AAA AGT
      C*                                      G*

                            30                                  40
Gly Lys Tyr Val Leu Gly Tyr Lys Gln Thr Leu Lys Met Ile Arg Gln Gly Lys Ala Lys Leu Val Ile
GGA AAG TAC GTG CTG GGG TAC AAA CAG ACT CTG AAG ATG ATC AGA CAA GGC AAA GCG AAA TTG GTT ATC
  G*                    C*      G*                                          G*

                50                                  60
Leu Ala Asn Asn Cys Pro Ala Leu Arg Lys Ser Glu Ile Glu Tyr Tyr Ala Met Leu Ala Lys Thr Gly
CTC GCC AAC AAC TGT CCA GCT TTG AGG AAA TCT GAA ATA GAA TAC TAT GCC ATG TTG GCT AAA ACT GGT
  T*                                                G*                                  G*
 70                                     80                                    90
Val His His Tyr Ser Gly Asn Asn Ile Glu Leu Gly Thr Ala Cys Gly Lys Tyr Tyr Arg Val Cys Thr
GTC CAT CAC TAC AGT GGC AAT AAC ATT GAA TTG GGC ACA GCG TGT GGA AAA TAC TAC AGA GTA TGC ACA
                                T*          C*

                            100                                 110             114
Leu Ala Ile Ile Asp Pro Gly Asp Ser Asp Ile Ile Arg Ser Met Pro Glu Gln Thr Gly Glu Lys
CTG GCT ATC ATT GAC CCA GGT GAT TCC GAT ATT ATT AGA AGC ATG CCA GAA CAG ACT GGT GAG AAG TAA
                                  T*

ACAAGAAAGTTCTCCTTT AATAAA ACTTTGCCAGAGCTCCTTTTAAAAA
         G*                                  ^
                                             G*
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Fig. 4. Nucleotide sequence and deduced aa sequence of pRL30 cDNA. Overlining indicates aa residues reported by Wool (1979). The nucleotides marked by asterisks are silent changes in mouse L30 (Wiedemann and Perry, 1984).

that there are twelve silent changes in the coding region. Eleven of these changes are at the third positions of the codons, as shown in Fig. 4. One of the changes is located at the first position of the leucine codon, the 80th codon from the N-terminus; T is present in the rat and C in the mouse. In the 3′ noncoding region of mouse L30 mRNA, a G is inserted between T and A located at the 44th and 45th positions, respectively, downstream from the termination codon of rat pRL30 (Fig. 4).

The aa sequence of L30 deduced from the nt sequence of the pRL30 insert is characterized by abundance of basic aa; about 16.7% are lysine, arginine and histidine, while about 7% are glutamic and aspartic acids. Assuming that the protein is not modified, its isoelectric point is estimated to be about 9.8. The lysine and arginine residues are concentrated in the N-terminal half while acidic aa residues are abundant in the C-terminal half, as in the case of pRS17-2 described above.

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