[原著] The Structure of Chicken Ribosomal Protein L30

The Structure of Chicken Ribosomal Protein L30

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ABSTRACT

The primary structure of the chicken ribosomal protein L30 was deduced from the sequence of nucleotide in a recombinant cDNA. Chicken ribosomal protein L30 has 115 amino acids and a molecular weight of 12,814 including initiator methionine. Hybridization of the cDNA to the restriction enzyme digests of chicken liver DNA suggests that L30 gene is a single copy. Chicken L30 is homologous to ribosomal proteins from other eukaryotes and archaebacteria but not to those from eubacteria. *Ryukyu Med. J., 14 (1) 43 ~ 48, 1994*

Key words : chicken, ribosomal protein L30, cDNA cloning, nucleotide sequence, amino acid sequence

INTRODUCTION

Ribosomes are cell organelles that participate in the translation of the information carried on the nucleotide sequence of mRNA into amino acid sequence of protein. Although their structure and composition are essentially conserved, eukaryotic ribosomal components have increased in numbers and sizes during evolution. Ribosomes in eukaryotic cells have 70 to 80 proteins and four molecules of RNA[1]. The biosynthesis of ribosomes requires the coordinate expression of the genes of these components. The mechanisms controlling the expression of these genes in eukaryotes are not known, although they have been studied in detail for *Escherichia coli* [2]. Fine structural analyses of the eukaryotic ribosomal protein genes are necessary to determine the mechanisms of the coordinate expression.

It is a prerequisite to have a cDNA probe for analyzing a specific gene which, in turn, may provide information about structure, function and evolution of the ribosomal proteins. Besides such biological interests, comparative studies on ribosomal proteins are expected to provide molecular accounts for the action of antibiotics, since many of them interact with either eukaryotic or prokaryotic ribosomal protein but not both. In this study, we isolated and determined the nucleotide sequence of a cDNA clone encoding chicken ribosomal protein L30. The structure of L30 was deduced from its nucleotide sequence. Computer analyses were performed to reveal its characteristics and evolution.

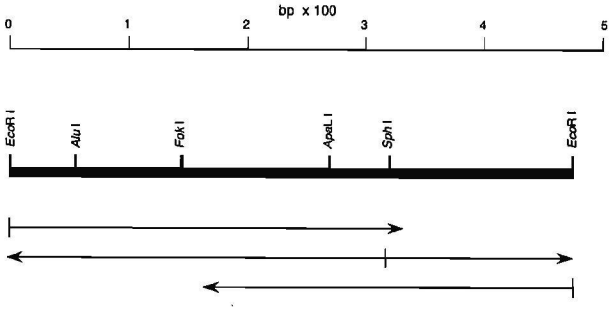

Fig. 1. Restriction endonuclease map of the cDNA insert and sequencing strategy. The map is oriented from 5' to 3' end relative to L30 mRNA. The directions of sequencing from restriction sites are indicated by arrows.

EXPERIMENTAL PROCEDURE

I. Materials

A λgt11 cDNA library derived from chicken embryo was purchased from Clontech Laboratories Inc. (U.S.A). Restriction endonucleases were obtained from Takara Shuzo (Japan). ECL gene detection system and [α-^{35}S]dATP (1000Ci/mmol) were from Amersham (UK). Sequenase version 2.0 DNA sequencing kit was a product of United States Biochemical Corp. (U.S.A.). Nitrocellulose membrane filter (BA 85) was obtained from Schleicher and Schuell (Germany).

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        10         20         30         40         50         60         70         80
AACGCCAGGCAGCGACAATGGTGGCCGCAAAGAAGACGAAAAAGTCCCTGGAGTCCATAAACTCCAGGCTTCAGCTGGTGATGAAAA
                  M  V  A  A  K  K  T  K  K  S  L  E  S  I  N  S  R  L  Q  L  V  M  K

 90        100        110        120        130        140        150        160        170
GCGGTAAATACGTGCTAGGATACAAACAGACTCTGAAGATGATTCGGCAAGGCAAAGCCAAGTTGGTCATCCTAGCCAACAACTGTC
S  G  K  Y  V  L  G  Y  K  Q  T  L  K  M  I  R  Q  G  K  A  K  L  V  I  L  A  N  N  C

   180        190        200        210        220        230        240        250        260
CTGCTTTGAGAAAATCGGAGATTGAATACTACGCCATGCTTGCAAAGACTGGTGTCCATCATTATAGTGGCAACAACATTGAATTGG
P  A  L  R  K  S  E  I  E  Y  Y  A  M  L  A  K  T  G  V  H  H  Y  S  G  N  N  I  E  L

       270        280        290        300        310        320        330        340        350
GCACAGCATGTGGAAAATACTACAGAGTGTGCACGCTGGCTATTATTGACCCAGGTGACTCTGACATCATTAGAAGCATGCCAGAAC
G  T  A  C  G  K  Y  Y  R  V  C  T  L  A  I  I  D  P  G  D  S  D  I  I  R  S  M  P  E

         360        370        380        390        400        410        420        430
AAACCAGTGAGAAGTAAATGCTGTAAAGGTGGTCTTTCTACAATAAAACTGGTTTTAGATCCATTTTAAGAAAAATGTGTATTGTAT
Q  T  S  E  K  *

 440        450        460
TATACTTCTGCTGGTTTTAGAGAACAAA
```

Fig. 2. Nucleotide sequence of the cDNA insert and the deduced amino acid sequence. Nucleotides are given numbers beginning at the 5' end of the cDNA insert. Deduced amino acid sequence is shown under the nucleotide sequence in one letter codes.

Table 1. Amino acid composition of chicken ribosomal protein L30

amino acids	residues	percent
Cystein	3	2.6
Aspartic acid	3	2.6
Asparagine	5	4.3
Threonine	6	5.2
Serine	9	7.8
Glutamic acid	6	5.2
Glutamine	4	3.5
Proline	3	2.6
Glycine	8	7.0
Alanine	9	7.8
Valine	6	5.2
Methionine	5	4.3
Isoleucine	9	7.8
Leucine	11	9.6
Tyrosine	7	6.1
Phenylalanine	0	0.0
Histidine	2	1.7
Lysine	14	12.2
Arginine	5	4.3
Tryptophan	0	0.0
Total	115	

II. cDNA Cloning

cDNA library was screened by ECL gene detection system using the insert of the plasmid pRL-30[3], which contains the cDNA of the rat ribosomal protein L30 as a probe. Each positive clone was purified through four cycles of the isolation procedure. The cDNA inserts of the positive clones were subcloned into the plasmid pUC18.

III. Nucleotide Sequence Determination

The nucleotide sequence of the cDNA insert in the recombinant pUC18 was determined directly by the dideoxynucleotide chain termination procedure[4] using Sequenase version 2.0 sequencing kit and [α -^{35}S]dATP. By subcloning *Sph*I fragments, overlapping sequences of both strands were determined as shown in Fig. 1.

IV. Southern Blot Analysis

Twenty μg each of chicken liver DNA was digested with the restriction enzyme *Hin*d III and *Kpn* I, respectively, separated by 1% agarose gel electrophoresis in TAE buffer (40mM Tris-acetate pH8.0, 1mM EDTA), vacuum-transferred to a nitrocellulose filter with 10 × SSC (1.5M NaCl, 0.15M sodium citrate) and probed with the cDNA using ECL gene detection system.

V. Computer programs

We used microcomputer to manipulate and analyze the nucleotide and amino acid sequences. The programs were written in C language and compiled with Microsoft C 6.0 compiler. Library search was made by FASTA program[5] kindly supplied by Dr. Pearson.

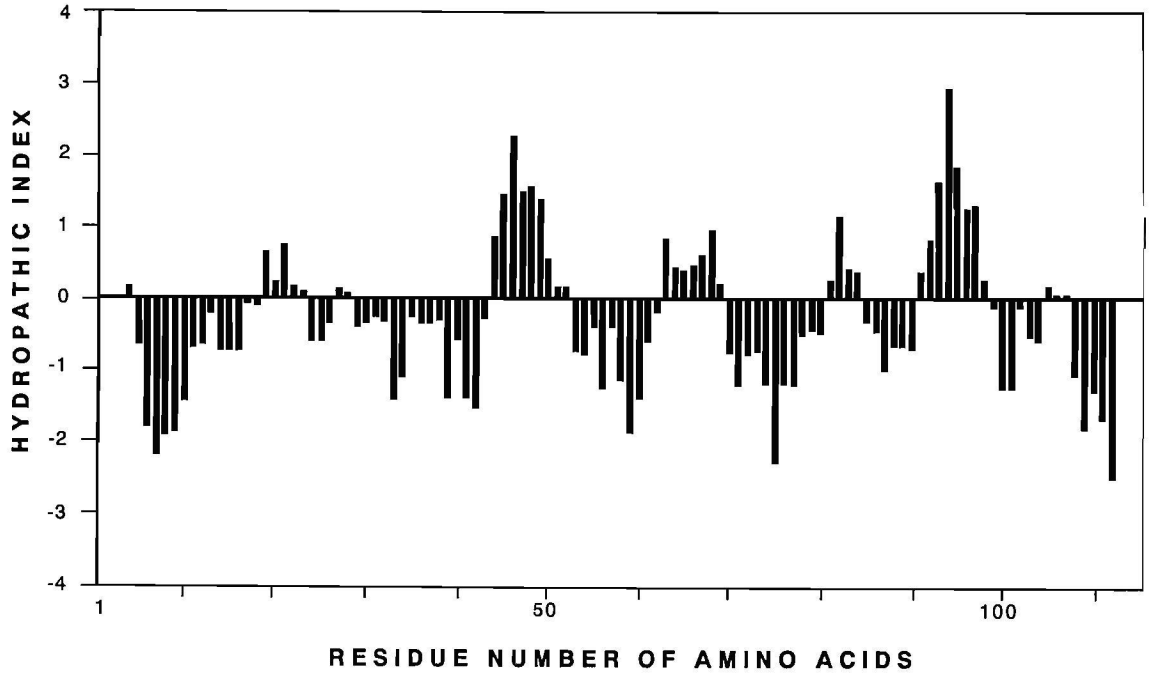

Fig. 3. Hydropathy profile of the protein L30. The profile was calculated using the parameters of Kyte and Doolittle[6] with span set at seven residues.

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1     MVAAKKTKKSLESINSRLQLVMKSGKYVLGYKQTLKMIRQGKAKLVILAN
      hhhhhhhhhhhhhhhhheeeeeeeteeeeeeeeeeeehhhhhhhhhhh

51    NCPALRKSEIEYYAMLAKTGVHHYSGNNIELGTACGKYYRVCTLAIIDPG
      hthchhhhhhhhhhhhheeeeccttteeeeeeettteeeeeeeettc

101   DSDIIRSMPEQTSEK
      ctteeeehhhhhhhh
```

Fig. 4. Secondary structure prediction for ribosomal protein L30. The secondary structure of the ribosomal protein L30 was predicted from the deduced amino acid sequence by the method of Garnier *et al.*[7]. Symbols are h: α-helix, e: extended sheet, t: β-turn and c: random coil.

RESULTS

I. cDNA Cloning

Screening about $2X10^4$ plaques, we obtained six positive clones. Each clone was purified through four cycles of isolation procedure. After three cycles of purification, all plaques showed positive signal indicating that each clone was practically pure.

II. Nucleotide Sequence

All the isolated clones had cDNA insert of the same length. Hence, we determined the nucleotides sequence of two clones. They were entirely the same and shown in Fig. 2 with the encoding amino acid sequence. Seventy nine percent of the nucleotides of chicken cDNA are the same as those of rat cDNA used as a cloning probe. When the amino acid sequence was compared with that of rat[3], only one amino acid out of 115 was changed; ^{113}Gly in rat to ^{113}Ser in chicken.

III. Characterization of the Protein

The molecular weight of chicken ribosomal protein L30 was calculated to be 12,814 from the sequence of amino acids including N-terminal methionine. It is not certain whether it is retained or removed after translation. The pI value was estimated to be 9.8. As shown in Table 1, the protein is specifically rich in the basic amino acid lysine. To obtain information on the higher structure of the protein L30, we performed some computer analyses of the protein. The hydropathy profile was calculated by the method of Kyte and Doolittle[6] and shown in Fig. 3. The secondary structure shown in Fig. 4 was predicted

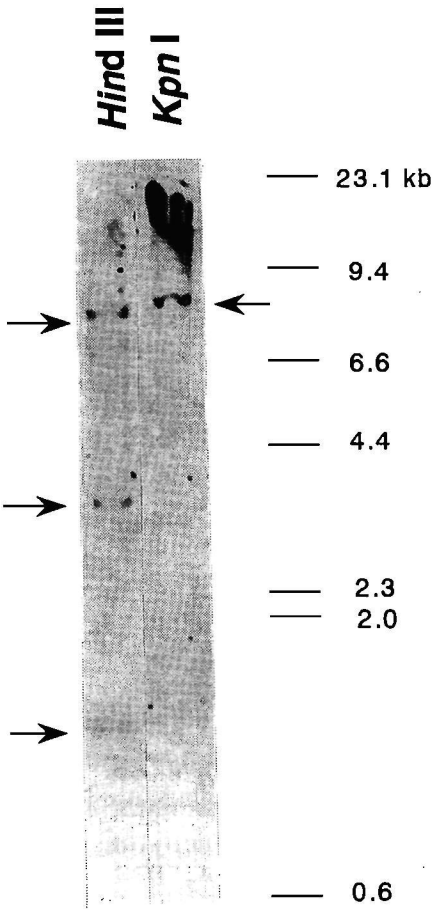

Fig. 5. Southern blot analysis of chicken genome. About 20 μg each of chicken liver DNA was analyzed as described in the experimental procedure section. The scale at the right hand indicate the positions of markers and their size. Arrows indicate the positions of hybridization bands.

by the algorithm of Garnier *et al.*[7].

IV. The Number of Copies of the L30 Gene

Chicken liver DNA was digested with *Hind* III and *Kpn* I, separated by electrophoresis and transferred to a membrane filter. The L30 gene was detected by hybridization with the cDNA (Fig. 5). A single band was observed for *Kpn* I digest and three bands for *Hind* III digest. Since these bands were at the same positions as the genomic clone, it was concluded that the chicken has a single copy of L30 gene.

V. Comparison of the Sequence of Amino Acids in Chicken L30 with ribosomal proteins from Other Species

The sequence of amino acids in chicken ribosomal protein L30 was compared, using the computer program FASTA[5], to the sequences of amino acids of more than 1,000 other ribosomal proteins contained in a library that we have compiled. Besides rat L30[11], which was used as the cloning probe, yeast ribosomal protein L32[8] and *Methanococcus vannielii* L30[9] were found to be homologous. The search into the EMBL data library uncovered some other archaebacterial counterparts registered as unidentified reading frames. These homologous proteins are aligned in Fig. 6. Alignment scores was calculated by the method of Dayhoff *et al.*[10] and shown in Table 2.

DISCUSSION

We have analyzed a cDNA clone encoding chicken ribosomal protein L30. The cDNA contains the entire coding sequence, although it lacks poly(A) tail. The primary structure of L30 was deduced from the nucleotide sequence. The structure of L30 thus determined is typical of ribosomal protein: small sized basic protein. The basic amino acid lysine is abundant in L30, but the other basic amino acid arginine is not.

Southern blot analysis showed that L30 gene is a single copy in the chicken genome. Many mammalian ribosomal protein genes have been reported to be in multiple copies[11-14], although no instance has been shown that more than one of them is functional. Chicken ribosomal protein genes, so far analyzed are however, a single copy (Maeda *et al.*, unpublished observation). The existence of many processed pseudogenes must be the characteristic of mammals as shown by Kuzumaki *et al.*[14] for ribosomal protein L35a family.

We found several proteins of mammals, yeast and archaebacteria homologous to chicken L30. However, we could not reveal any counterpart of chicken L30 in eubacteria, although we used the computer program ALIGN[10] that is powerful enough to detect even a minor similarity in sequences, irrespective of the fact that all of the ribosomal proteins of *Escherichia coli* have been sequenced[15]. It is not irrelevant to assume that eubacteria do not have the counterpart of eukaryotic L30. Since eukaryotic ribosomes have about 80 proteins instead of 55 of prokaryotic ribosomes[1], L30 may be one of the extra proteins of eukaryotes. There has been no description on the function of L30. It is reasonable, however, to suppose that the protein is working not directly on the formation of peptide bond but on the fine control of ribosomal function.

Ribosomal proteins migrate to the nucleolus after biosynthesis in the cytoplasm and are assembled into ribosomal particles. It is believed that a short amino acid sequence in a ribosomal protein signals its migration to the nucleolus. Schaap *et al.*[16] proposed a signal sequence for nuclear localization of a yeast ribosomal protein L25, but the sequence is not common to all eukaryotic ribosomal proteins. Since our experimental approach (Quaye *et al.* unpublished observation) suggests that a cluster of basic amino acids is crucial, we suppose that the nucleolar transport signal in L30 may be a short sequence containing $R^{56}K^{57}$, which is conserved among eukaryotes but

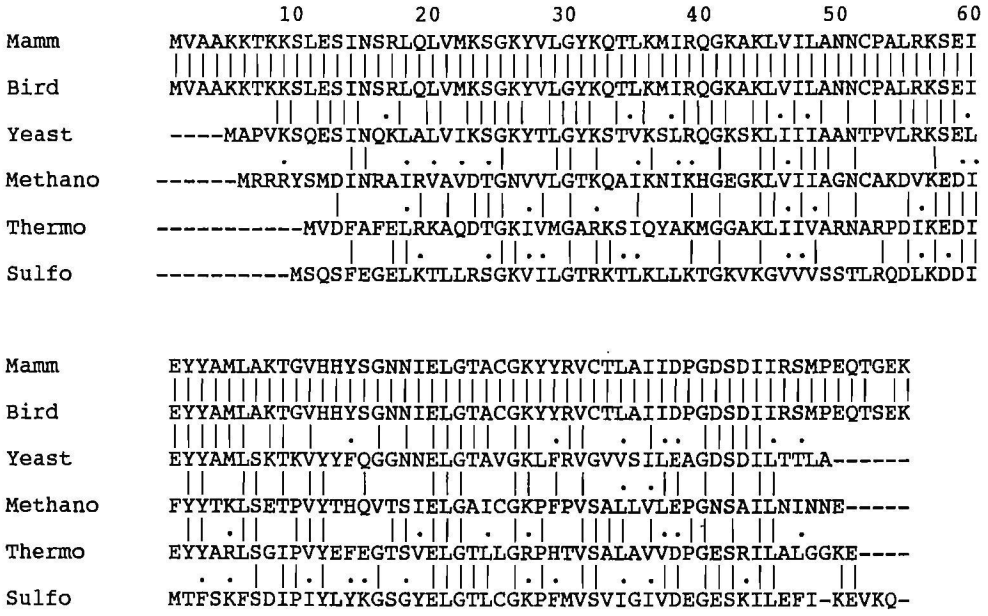

Fig. 6. Alignment of homologous proteins. The proteins homologous to chicken L30 are aligned. '|' and '.' indicate the same and similar amino acids, respectively. Mamm, rat and mouse; Bird, chicken; Yeast, *Saccharomyces cerevisiae*; Methano, *Methanococcus vannielii*; Thermo, *Thermococcus celer* ; Sulfo, *Sulfolobus acidocaldalius*.

Table 2. Comparison of the sequences of amino acids in a set of related ribosomal proteins

	Mamm	Bird	Yeast	Methano	Thermo	Sulfo
Mamm		34.6	24.3	15.5	12.6	13.1
Bird			24.3	15.3	12.6	13.3
Yeast				13.6	11.6	11.3
Methano					17.7	15.2
Thermo						15.8
Sulfo						

The component proteins are rat and mouse L30 (Mamm), chicken L30 (Bird), *Saccharomyces cerevisiae* L32 (Yeast), *Methanococcus vannielii* L30 (Methano), *Thermococcus celer* (Thermo) and *Sulfolobus acidocaldalius* (Sulfo). The comparisons were made by the algorithm of Dayhoff *et al.*[15]. The values are the number of standard deviations of the real score above the random score; 3 standard deviations correspond to $p < 0.001$ and is usually taken to indicate that the proteins are likely to be homologous.

not in archaebacteria (Fig. 6). However, much more experimental works and sequence data are required for establishing the signal sequence of ribosomal proteins.

Our primary purpose was to obtain a cDNA probe for gene cloning. However, the information described here may also contribute to unraveling the function of ribosomal proteins, in understanding the evolution of ribosomes and uncovering the amino acid sequences that direct the proteins to the nucleolus for assembly on nascent rRNA.

ACKNOWLEDGEMENT

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