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The primary structure of rat ribosomal protein S8

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ABSTRACT

The amino acid sequence of rat ribosomal protein S8 was deduced from the sequence of nucleotides in a recombinant cDNA and confirmed from the NH_2- and carboxyl-terminal amino acid sequences of the protein. Ribosomal protein S8 contains 207 amino acids (the NH_2-terminal methionine is removed after translation of the mRNA) and has a molecular weight of 23,928. Hybridization of the cDNA to digests of nuclear DNA suggests that there are 7-9 copies of the S8 gene. Ribosomal protein S8 contains a possible internal repeat that has 12 or 13 residues, is basic, and occurs 5 times in the protein.

INTRODUCTION

It is assumed that a molecular account of the function of eukaryotic ribosomes will only follow from knowledge of the structure and that this, in turn, requires information on the chemistry of the constituent proteins and nucleic acids. This inventory of data is necessary also for the second order structural problem, namely, the analysis of the chemistry of the interactions of the proteins with the nucleic acids. Progress has been made in this endeavor, although, a good deal remains to be done (1). The covalent structure of the four species of RNA in rat ribosomes has been established (2-7). In addition, eighty-four proteins have been isolated from the particles (8) and the sequence of amino acids in several has been determined directly (9-13). The latter task is being expedited now by the application of recombinant DNA technology. Thus, the structure of a number of rat ribosomal proteins has been deduced from the sequence of nucleotides in recombinant cDNAs (14-21). We report here the structure of rat ribosomal protein S8 which we have inferred from the sequence of nucleotides in a recombinant cDNA and which we have confirmed by sequencing portions of the protein. Ribosomal protein S8 can be crosslinked to eukaryotic initiation factor eIF-3 (22, 23); furthermore, the protein has been located at the interface between ribosomal subunits (24). These observations imply that protein S8 forms part of the domain concerned with the binding of initiation factors to the 40S subunit at the start of the translation of mRNA.

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EXPERIMENTAL PROCEDURES

Preparation of Recombinant cDNAs Encoding Rat Ribsomal Protein S8. The recombinant DNA procedures and the methods used to determine the sequence of nucleotides in the nucleic acid were either described or cited before (20). The strategy that was used to design a probe for the cDNA encoding rat ribosomal protein S8, based on the sequence of 6 amino acids near the NH_2 terminus of the protein has been reported (20). The probe, a mixture of 32 different oligodeoxynucleotides, 17 nucleotides in length, was synthesized on a solid support by the methoxyphosphoramidite method using an Applied Biosystems, Model 380B, DNA synthesizer (25) and the oligonucleotides were purified by polyacrylamide gel electrophoresis.

Rat ribosomal protein S8 cDNA was hybridized to restriction enzyme digests of genomic DNA (26).

Comparison of the Amino Acid Sequences of Ribosomal Proteins. The computer program, RELATE (27), was used to assess possible evolutionary relationships between S8 and other ribosomal proteins. The scoring matrix was Dayhoff's MDM '78 (27).

RESULTS AND DISCUSSION

The Sequence of Nucleotides in a Recombinant cDNA Encoding Rat Ribosomal Protein S8. A cDNA library of 30,000 independent transformants was constructed from poly(A)$^+$mRNA prepared from regenerating rat liver (20). A random selection of 14,000 colonies from the library was screened for clones that hybridized to an oligonucleotide probe that was synthesized to be complementary to the sequence of nucleotides predicted to be present in the portion of the mRNA that encoded 6 amino acids (-Asp-Asn-Trp-His-Lys-Arg-) near the NH_2 terminus of rat ribosomal protein S8. Seven colonies gave a positive hybridization signal with the probe. The DNA from the plasmids of the 7 transformants was isolated, digested with restriction endonucleases, and analyzed by gel electrophoresis. These clones had inserts that ranged in length from 0.45 to 0.7 kilobases and Southern blot hybridization with the oligonucleotide probe confirmed that all of the inserts contained cDNA for S8. The anticipated length of the S8 coding sequence, calculated from the molecular weight of the protein, is 670 nucleotides. The clone with the largest insert, pS8-14, was selected and the nucleotide sequence of the cDNA was determined. The sequences of nucleotides from both strands of the DNA, and overlapping sequences for each restriction site, were obtained.

The cDNA insert in pS8-14 contains 735 nucleotides and includes the 5' homopolymer linkers, the 5' noncoding sequence, and a single open reading frame (Fig. 1). In the other two reading frames the sequence is interrupted by many termination codons. The open reading frame of 627 nucleotides begins at an ATG codon at position

```
                                        30                                                  60                                                  90
GGG GGG GGG GGG GGG GGG GCT CTT TCC AGC CAG CGC CGA GCG ATG GGC ATC TCT CGG GAC AAC TGG CAC AAG CGC CGC AAG ACC GGG GGT
                                                                MET GLY ILE SER ARG ASP ASN TRP HIS LYS ARG ARG LYS THR GLY GLY
                                                                 1                                                  10

                                       120                                                 150                                                 180
AAG AGA AAA CCC TAC CAC AAG AAG CGG AAG TAT GAG CTG GGA CGG CCG GCC GCC AAC ACT AAG ATT GGC CCT CGC CGC ATA CAT ACA GTC
LYS ARG LYS PRO TYR HIS LYS LYS ARG LYS TYR GLU LEU GLY ARG PRO ALA ALA ASN THR LYS ILE GLY PRO ARG ARG ILE HIS THR VAL
            20                                                  30                                                  40

                                       210                                                 240                                                 270
CGA GTT CGA GGA GGC AAT AAG AAG TAT CGT GCT CTG AGA TTG GAT GTG GGG AAC TTT TCC TGG GGC TCA GAG TGT TGT ACT CGC AAA ACA
ARG VAL ARG GLY GLY ASN LYS LYS TYR ARG ALA LEU ARG LEU ASP VAL GLY ASN PHE SER TRP GLY SER GLU CYS CYS THR ARG LYS THR
            50                                                  60                                                  70

                                       300                                                 330                                                 360
AGG ATC ATT GAT GTT GTC TAC AAT GCA TCC AAT AAC GAG CTT GTC CGC ACC AAG ACC CTG GTG AAG AAC TGC ATT GTG CTT ATT GAC AGC
ARG ILE ILE ASP VAL VAL TYR ASN ALA SER ASN ASN GLU LEU VAL ARG THR LYS THR LEU VAL LYS ASN CYS ILE VAL LEU ILE ASP SER
            80                                                  90                                                  100

                                       390                                                 420                                                 450
ACA CCG TAC CGA CAG TGG TAC GAG TCC CAC TAT GCA CTG CCC CTG GGC CGC AAG AAG GGG GCC AAG CTG ACT CCT GAG GAG GAA GAG ATT
THR PRO TYR ARG GLN TRP TYR GLU SER HIS TYR ALA LEU PRO LEU GLY ARG LYS LYS GLY ALA LYS LEU THR PRO GLU GLU GLU GLU ILE
            110                                                 120                                                 130

                                       480                                                 510                                                 540
TTA AAC AAA AAA CGA TCA AAG AAA ATT CAG AAG AAA TAT GAT GAA AGG AAA AAG AAT GCC AAA ATC AGC AGT CTT CTG GAG GAG CAG TTC
LEU ASN LYS LYS ARG SER LYS LYS ILE GLN LYS LYS TYR ASP GLU ARG LYS LYS ASN ALA LYS ILE SER SER LEU LEU GLU GLU GLN PHE
            140                                                 150                                                 160

                                       570                                                 600                                                 630
CAG CAG GGC AAG CTT CTC GCC TGT ATT GCC TCA AGA CCA GGC CAG TGT GGC AGA GCA GAT GGC TAT GTG CTC GAA GGC AAG GAG CTG GAG
GLN GLN GLY LYS LEU LEU ALA CYS ILE ALA SER ARG PRO GLY GLN CYS GLY ARG ALA ASP GLY TYR VAL LEU GLU GLY LYS GLU LEU GLU
            170                                                 180                                                 190

                                       660                                                 690                                                 720
TTC TAT CTG CGG AAG ATC AAA GCC CGG AAA GGC AAA TAA ACT GTC ATA GCT CGT GTA ATA AAG GTG TTT GCT GTT CTG TAT ATG TAA AAA
PHE TYR LEU ARG LYS ILE LYS ALA ARG LYS GLY LYS END
            200

AAA ACC CCC CCC CCC
```

Fig. 1. The sequence of nucleotides in the cDNA insert in plasmid pS8-14 and the amino acid sequence encoded in the open reading frame. The nucleotide sequence in the cDNA insert encoding rat ribosomal protein S8 was converted to an amino acid sequence with the computer program of Queen and Korn (41). The position of the nucleotides in the cDNA insert in pS8-14 is given above the residue; the position of amino acids in protein S8 is designated below the residue.

43 and ends with a termination codon (TAA) at position 667; it encodes 208 amino acids (Fig. 1). The hexamer AATAAA, presumed to be the recognition sequence directing post-transcriptional cleavage-polyadenylation of the 3' end of pre-mRNA (28), is located 23 nucleotides upstream of the start of the poly(A) stretch. This hexamer is generally found 10 to 30 nucleotides from the site of the initiation of polyadenylation. The length of the poly(A) tail is 9 nucleotides. The 5' noncoding sequence is 23 nucleotides in length. The context in which the initiation codon occurs, GCGATGG, differs from the optimum, ACCATGG (29).

The first nucleotide of the S8 cDNA (position 20 in Fig. 1; position -23 if the A in the initiation codon is taken as $^{+}1$) is a cytosine and it is followed by a sequence of 8 consecutive pyrimidines i.e., CTCTTTCC. Pyrimidine stretches have been reported to be present in the 5' untranslated region of many eukaryotic ribosomal protein mRNAs (18, 30-33). The near ubiquity of this track of pyrimidines suggests that it plays a role in the regulation of the translation of at least some of the mRNAs for eukaryotic ribosomal proteins.

TABLE I. Amino acid composition of rat ribosomal protein S8

Amino Acids	A	B
Alanine	11	11
Arginine	20	23
Aspartic acid and Asparagine	15	6 + 10
Cysteine	-	5
Glutamic acid and Glutamine	20	14 + 6
Glycine	20	17
Histidine	4	4
Isoleucine	10	12
Leucine	16	17
Lysine	27	31
Methionine	0.5	1[a]
Phenylalanine	3	3
Proline	6	7
Serine	10	10
Threonine	8	9
Tryptophan	-	3
Tyrosine	8	10
Valine	10	9
Residues		208

[a]The NH_2-terminal methionine is removed after translation of the mRNA.

The amino acid composition (in numbers of residues) was determined either (A) from an analysis of an hydrolysate of purified S8 (34) or inferred (B) from the sequence of nucleotides in a recombinant cDNA.

The Primary Structure of Rat Ribosomal Protein S8. The reading frame in pS8-14 flanked by initiation and termination codons specifies a protein of 208 amino acids (Fig. 1). This protein was identified as rat ribosomal protein S8 in the following manner: The recombinant cDNA clone (pS8-14) was selected using an oligonucleotide probe that was complementary to the codons for a sequence of 6 amino acids near the NH_2 terminus of S8. The amino acid composition inferred from the cDNA is very close to that previously derived (34) from an hydrolysate of purified S8 (Table I). The sequence of amino acids deduced from the sequence of nucleotides in pS8-14 corresponds to the NH_2-terminal 20 residues (35) and to the carboxyl-terminal 4 residues, -Arg-Lys-Gly-Lys, determined directly from protein S8 by digestion with carboxypeptidases A and B (data not shown).

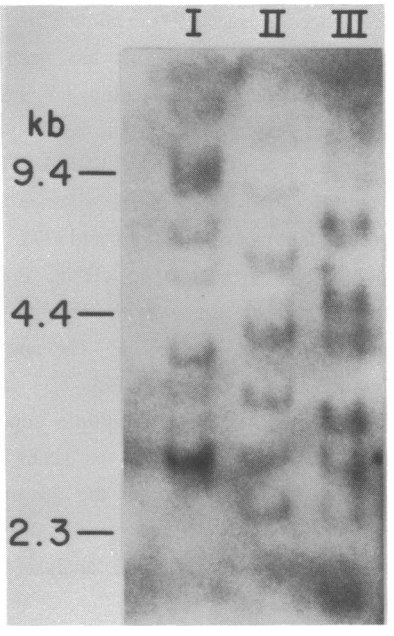

Fig. 2. Hybridization of ribosomal protein S8 cDNA to rat genomic DNA. Rat nuclear DNA (10 μg) was disgested with restriction enzymes: BamHI (lane I); HindIII (lane II); or EcoRI (lane III). The digests were resolved by electrophoresis in 0.7% agarose gels and transferred to GeneScreen Plus nylon filters. Uniformly labeled radioactive S8 cDNA insert from pS8-14 was hybridized to the immobilized genomic DNA. The position to which DNA standards of the size designated (in kilobase pairs) migrate is shown to the left.

The molecular weight of rat ribosomal protein S8, calculated from the sequence of amino acids deduced from the nucleotide sequence of pS8-14 is 24,059. However, the NH_2-terminal methionine encoded in the S8 mRNA is removed after translation since it is not found in the amino acid sequence derived from the protein (35). Thus, the number of residues in the mature protein is 207 and the molecular weight is 23,928, close to that of 26,800 estimated from the migration of the purified protein in sodium dodecyl sulfate gels (34).

Protein S8 has an excess of basic (23 arginyl, 31 lysyl, and 4 histidyl) over acidic (6 aspartyl and 14 glutamyl) residues; a total of 58 or 28% of the former and 20 or 10% of the latter. There are 5 cysteinyl residues (at positions 71, 72, 100, 174, and 182) but whether they are linked in disulfide bridges is not known. The basic and acidic residues are not uniformly or randomly distributed in S8. There are 2 groups of 5 consecutive basic amino acids near the NH_2 terminus of S8 (at positions 9-13 and 22-

26); and there are 4 places where 3 successive residues are basic. The clustering of basic amino acids in eukaryotic ribosomal proteins has been remarked on before (20, 26, 30, 36) but the significance of the observation, if indeed there is any, is not known. Twelve of the 20 acidic residues are in the carboxyl-terminal one-third of S8; this includes a stretch of 4 glutamyls at position 132-135 (Fig. 3). The most striking feature of the amino acid composition of S8 is the absence of methionine; S8 is the only mammalian ribosomal protein that lacks this amino acid (37).

The Number of Copies of the S8 Gene. The cDNA insert in pS8-14 was made radioactive and used to probe digests made with restriction endonucleases (BamHI, EcoRI, or HindIII) from rat liver nuclear DNA (26). The number of hybridization bands suggest that there are 7-9 copies of the S8 gene (Fig. 2). Other mammalian ribosomal protein genes have been found to be present in multiple copies (38). However, in no instance has it been shown that more than one of the genes is functional (30, 31, 39). The presumption is that for each ribosomal protein the genome contains only one gene that is expressed, that the other copies are non-functional pseudogenes. It needs to be noted that this presumption derives from the analysis of a limited number of families and is at best a tentative conclusion.

Comparison of the Sequence of Amino Acids in Rat S8 and in Ribosomal Proteins from Other Species. Comparison of the sequences of amino acids in ribosomal proteins may inform us concerning the details of their evolution. In addition, identification of conserved amino acid sequences may provide clues to the function of the proteins, to the structure of the sites that associate with the rRNAs, and to the sequences of amino acids that direct ribosomal proteins from the cytoplasm to the nucleus. Finally, these comparisons may inform us as to why the number of proteins has increased from the 52 contained in prokaryotic ribosomes to the 70 to 80 that are found in eukaryotes; an increase, incidently, that has occurred without apparent significant changes in the reactions that the particles catalyze. For these reasons, the sequence of amino acids in rat ribosomal protein S8 was compared, using the computer program RELATE (30), to the sequence of amino acids in more than 250 other ribosomal proteins contained in a library that we have compiled, and that includes the complete set of 52 from Escherichia coli (40). The only comparison to yield a significant score was with rat ribosomal protein S6 (3.7 standard deviation units), a relationship we had noted before (26). However, except for one short segment we do not find anything significant in the alignment of the two proteins. We note, however, that there is a segment of 8 consecutive identical amino acids, LEGKELEF, that occurs at position 190-197 in rat ribosomal protein S8 and at position 147-154 in E. coli ribosomal protein S1 (40).

Possible Repeat Sequences in Rat Ribosomal Protein S8. The sequence of amino acids

$_{9}$H	K	R	R	K	T	G	G	K	R	K	P	Y
$_{22}$H	K	K	R	K	Y	E	L	-	G	R	P	A
$_{44}$H	T	V	R	V	R	G	G	N	K	K	-	Y
$_{72}$C	T	R	K	T	R	I	I	D	V	V	-	Y
$_{138}$N	K	K	R	S	R	K	I	Q	K	K	-	Y
	H_3	B_3T_2	B_4	B_5	B_2	B_3	G_2	G_2I_2	X	B_3	B_4	P_2	Y_4

Fig. 3. A possible repeat of a sequence of 12 or 13 amino acids in rat ribosomal protein S8. The subscripts preceeding the putative repeats designates the position of the residue in the amino acid sequence of protein S8. A consensus sequence for the repeats is given at the bottom; the frequency with which an identical or related (arginine and lysine) residue occurs at the same position is designated by a subscript to the residue; arginine and lysine are given together as B for basic.

in S8 was searched for internal duplications. A segment of 5 basic residues, HKRRK, at position 9-13 is repeated, HKKRK, at position 22-26; the only deviation from identity is the conservative substitution of a lysine for an arginine in the repeat (Fig. 1). This sequence is so short as to raise a question of its significance. However, the same sequence was found, using the computer program RELATE, to be part of what might be a larger internal duplication. This potential repeat is of 12 or 13 residues and occurs 5 times, i.e. at positions 9, 22, 44, 72, and 138 (Fig. 3). The sequences have 3-8 basic amino acids each, indeed, 29 of the 61 residues in the 5 repeats are basic and there are only two acidic residues. It is not possible to establish the statistical significance of the relatedness of the 5 putative repeats, however, the similarity is noteworthy. If we accept arginine and lysine as equivalent amino acids then at 8 of the 13 positions the same residue occurs in at least 3 of the 5 repeats, moreover, at 4 of the 5 remaining positions there are like residues in two of the segments (Fig. 3).

We had found before (19) that rat ribosomal protein L7 has 5 repeats of a segment of 12 amino acids arranged in tandem near the NH_2 terminus of the protein. The repeats in L7 are very basic. Rat ribosomal protein S6 also has related repeated sequences of 10 amino acids that occur at 4 separate positions and that are very basic (26). The occurance of multiple, related, basic repeats in another ribosomal protein implies that they have functional significance, but we do not as yet have any indication what this might be. Possibilities that suggest themselves are that they play a role in the interaction with RNA (ribosomal, transfer, or messenger) or that they are

involved in directing the proteins to the nucleolus for assembly of ribosomes. There is evidence that information specifying the localization of proteins in the nucleus is encoded in short sequences of amino acids (42), although, it is not yet possible to derive a consensus sequence nor to formulate general rules for the structure of the peptide. The best characterized nuclear localization peptide is in SV40 large T antigen; it has the sequence, PKKKRKV (43). Yeast ribosomal protein L3 has the related sequence, PKRK, in a stretch of 21 amino acids at its NH_2 terminus that is essential for translocation to the nucleus (44). In these two examples, entry into the nucleus is contingent on a consecutive sequence of several basic amino acids preceeded by a prolyl residue. The repeat in rat S8 has a prolyl residue in only 2 of the 5 repeats and it is near the carboxyl terminus. Although, there is no experimental evidence available to evaluate the proposal the structure of the repeats in these rat ribosomal proteins have sufficient similarity to the known nuclear localization sequences as to require consideration of the possibility that they serve the same function.

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