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Emetine Resistance of Chinese Hamster Cells: Structures of Wild-Type and Mutant Ribosomal Protein S14 mRNAs†

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The Chinese hamster ovary (CHO) cell 40S ribosomal subunit protein S14 provides a unique opportunity to investigate an important mammalian housekeeping gene and its mRNA and protein products. The S14 gene appears to be single copy, and CHO cell S14 mutants have been isolated as emetine-resistant (*emtB*) clones in tissue culture. Thus, S14 is the only mammalian ribosomal protein whose gene structure and function are amenable to straightforward genetic and biochemical analysis. Recently, we isolated a wild-type Chinese hamster lung cell cDNA clone, pCS14-1, including an almost complete copy of the ribosomal protein S14 message (N. Nakamichi, D. D. Rhoads, and D. J. Roufa, J. Biol. Chem. 258:13236–13242, 1983). Here we describe comparable cDNAs from wild-type and *emtB* CHO cells. We report both mRNA and polypeptide sequences of the wild-type and mutant ribosomal protein transcripts. As a consequence of the genetic methods used to obtain our emetine-resistant mutants, the *emtB* S14 cDNAs differ from wild-type cDNA by single-base changes. Physical and chemical features of polypeptides encoded by the cDNAs are consistent with well-characterized S14 protein polymorphisms. The three *emtB* mutations analyzed affect two adjacent arginine codons within the very basic S14 carboxyl region, indicating a significant role for this portion of the protein in the function and architecture of the mammalian 40S ribosomal subunit.

Among mammalian ribosomal protein genes, the Chinese hamster ovary (CHO) cell locus specifying 40S subunit protein S14 is particularly suited to direct biochemical and genetic analysis. S14 is the only mammalian ribosomal protein gene for which both mutants and molecular probes are available. We have identified protein S14 as the product of the CHO cell emetine resistance locus *emtB* (1, 2, 13, 14), and we have described bacterial plasmids containing Chinese hamster lung (CHL) cell S14 cDNAs (18). To characterize this ribosomal protein gene and its *emtB* alleles, we have used a CHL cDNA (pCS14-1) to isolate wild-type and mutant CHO cell S14 cDNAs.

In the present study, we determined the CHO S14 cDNA sequence and precise nucleic acid and protein alterations affected by several *emtB* mutations. Only a few single-base substitutions distinguish mutant from wild-type cDNAs. Resulting amino acid replacements account for electrophoretic variants of S14 protein associated with *emtB* mutations (1, 2, 13, 14). Our data indicate that the carboxyl region of the S14 protein is an important determinant of 40S ribosomal subunit structure and function.

MATERIALS AND METHODS

Cell lines, microbial strains, and materials. The wild-type and mutant Chinese hamster cell lines and methods used to maintain them in tissue culture have been described previously (1, 2, 13, 14, 18). Procedures for isolating Chinese hamster polyadenylated mRNA and for cloning cDNAs into pBR322 and M13 vectors also have been described before (14, 18).

[α-^{32}P]deoxyribonucleoside triphosphates (800 to 900 Ci/mmol) and [^{35}S]methionine (1,200 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Unlabeled deoxyribonucleoside, dideoxyribonucleoside triphosphates, and M13 "17mer" primer were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. Restriction endonucleases, oligodeoxyguanylate-tailed, *Pst*I-cleaved pBR322, *Escherichia coli* DNA polymerase I (Klenow fragment), T4 DNA ligase, RNase T1, and RNase H were from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Avian myeloblastosis virus reverse transcriptase was a product of Life Sciences, Inc., St. Petersburg, Fla. All of these enzymes were used as suggested by the suppliers.

Molecular cloning and DNA sequencing. Mutant S14 cDNAs were synthesized according to the method of Gubler and Hoffman (9) from size-fractionated, polyadenylated mRNAs (18). Double-stranded cDNAs were treated with RNase T1 and cloned in the *Pst*I site of pBR322 as described previously (18). Recombinant plasmids were screened with purified pCS14-1 cDNA as a hybridization probe. Clones recognized were digested with *Pst*I, and resulting DNA fragments were purified by polyacrylamide gel electrophoresis (15, 18). Restriction fragments derived from the cDNAs were subcloned into M13mp10 and M13mp11 vectors in both orientations for DNA sequence determinations (see Fig. 1) (16). All recombinant DNA procedures were performed under P2 containment in compliance with National Institutes of Health guidelines for recombinant DNA research.

DNA sequences were determined by the dideoxynucleoside chain termination method (23) with M13 17mer primer and *E. coli* DNA polymerase I (Klenow fragment). They were analyzed using the SeqAid package of programs developed by the authors for IBM PC and IBM PC-compatible microcomputers. These programs are available from us on request by sending a 5.25-in floppy diskette with a prepaid, self-addressed diskette mailer.

RESULTS

Sequence of wild-type Chinese hamster S14 cDNA. A restriction endonuclease map of Chinese hamster cell ribosomal protein S14 cDNA, indicating the orientation of

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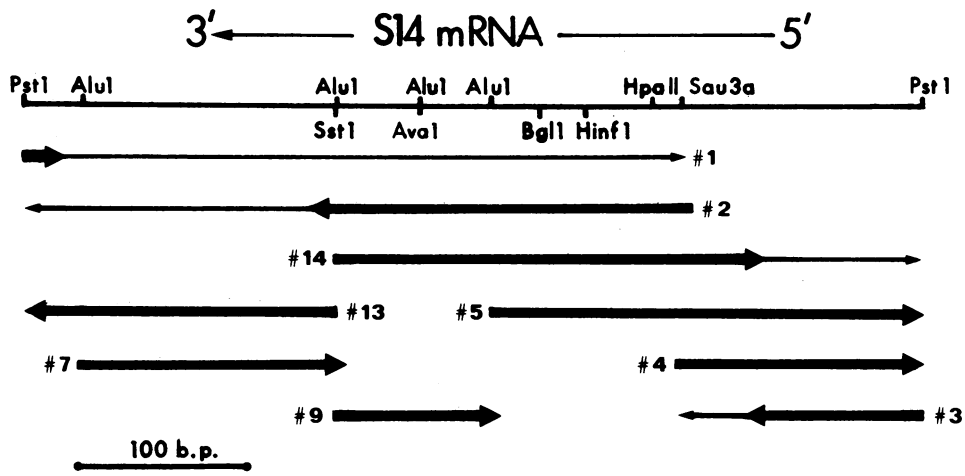

FIG. 1. Restriction endonuclease map of pCS14-11 cDNA and strategy for determining its sequence. At the top of the figure is a restriction site map of pCS14-11 cDNA specifying the orientation of S14 mRNA (18). Restriction endonuclease cleavage sites indicated above the map were known from previous studies (18). Sites depicted below the map were recognized through DNA sequence determination. The locations and 5′-to-3′ polarities of M13 subclones used to determine the cDNA sequence are illustrated below the restriction map. Extents to which the sequence of each subclone was analyzed are indicated in boldface.

the mRNA with respect to the clone, is depicted at the top of Fig. 1. Cleavage sites shown above the map, as well as the 5′-to-3′ polarity of the mRNA, were known from a previous analysis of pCS14-1 (18). Restriction sites indicated below the map were recognized from the cDNA sequence itself and subsequently were used to confirm portions of the cDNA structure.

To obtain the nucleotide sequence of wild-type CHO cell S14 cDNA, we subcloned cDNA restriction fragments into the M13mp10 (subclones 1 and 3) and M13mp11 (subclones 2, 4, 5, 7, 9, 13, and 14) vectors. When possible, we employed fragments with nonidentical molecular ends to orient cDNAs within the M13 polylinker (16). The orientations of all of the clones were determined by hybridization with the single-stranded S14 probes described before (18). The M13 subclones sequenced are indicated in the lower portion of Fig. 1.

The wild-type CHO pCS14-11 sequence (Fig. 2) was determined from overlapping DNA fragments derived from both strands of the cDNA clone. As anticipated from the strategy used to synthesize the cDNAs (see above; 18), pCS14-11 cDNA is flanked by *Pst*I cleavage sites and deoxyguanylate:deoxycytidylate tails (not illustrated in Fig. 2). The cDNA includes four restriction endonuclease cleavage sites whose presence was not known before these studies: a *Hin*fI site (residue 202); a *Bgl*I site (residue 220); an *Ava*I site (residue 290) observed only in the S14 cDNA cloned from a CHL message, i.e., pCS14-1; and an *Sst*I site (residue 350). Locations of these sites were confirmed by restriction endonuclease mapping (not shown).

S14 cDNA contains 10 possible protein-coding sequences delimited by translational initiation and termination sites (Fig. 2). Neither the amino acid nor the nucleic acid sequences of a mammalian S14 ribosomal protein were known at the outset of these experiments. Thus, we could not identify the S14 translational reading frame in pCS14-11 by comparison with published sequences. However, substantial information about hamster and rat S14 proteins was available and permitted us to discern the correct S14 cDNA coding sequence. The Chinese hamster S14 protein is extremely basic (pI > 10) and exhibits a molecular weight of approximately 15,000 (1, 13). Of the 10 open reading frames in the cDNA, 9 would encode proteins substantially smaller than those ascribed to S14 and on this basis were eliminated from consideration. Only the 5′-most AUG codon (residue 1) initiates a translational reading frame long enough to encode the S14 protein. This reading frame extends from residue 1 to a single TGA terminator at residue 454 (Fig. 2). Amino acids encoded by the sequence are represented by standard single-letter abbreviations (see legend to Fig. 2).

The canonical polyadenylation signal (aataaa; residues 484 to 489 in Fig. 2) points to the polyadenylate sequence beginning at residue 517. Chinese hamster S14 mRNA is approximately 650 nucleotides long (18), 565 bases of which are included in pCS14-11 cDNA. Assuming that S14 mRNA contains a 3′-polyadenylate tail of 100 to 200 bases, pCS14-11 lacks very little, if any, of the nontranslated mRNA 5′ sequence.

Primary structure of Chinese hamster cell ribosomal protein S14. The S14 cDNA sequence encodes a very basic 151-amino-acid polypeptide whose residue molecular weight is 16,275. The amino acid composition of the protein is summarized and compared with the composition of rat liver S14 protein (5) in Table 1. Chinese hamster and rat liver S14 proteins exhibit nearly identical amino acid compositions. Both are extremely rich in glycine, alanine, and arginine, and both are deficient in tryptophan and tyrosine. The abundance of arginine and lysine and the average contents of glutamic and aspartic acids are consistent with the basic character of the S14 protein. Interestingly, CHO S14 Arg and Lys residues are concentrated in both termini of the protein. The 10 amino-terminal residues include four lysines and one arginine. The 11 carboxyl-terminal amino acids include six arginine residues and one lysine residue. Only the apparently higher content of methionine (2.6 mol%) in the Chinese hamster protein distinguishes it from the rat liver ribosomal protein (1.0 mol%). However, because the rat liver S14 amino acid composition was determined by acid hydrolysis (5), it is possible that its methionine content was underestimated. Peptide analysis (4, 7) of the cell-free translation product encoded by purified S14 mRNA (18) indicated that Chinese hamster S14 protein yields four methionine-containing tryptic peptides, a result consistent with the sequence depicted in Fig. 2.

Primary structures of mutant S14 cDNAs. We have demonstrated that ethyl methanesulfonate-induced CHO cell *emtB* mutants attain emetine-resistance phenotypes via somatic mutations in the S14 structural gene (1, 2, 18). The mutants comprise two classes: one-step (single-site) mutants resistant to low concentrations of emetine (2×10^{-7} M) and two-step (multisite) mutants resistant to 2×10^{-6} M emetine (13). Both classes elaborate polyadenylated mRNAs that encode electrophoretically altered S14 proteins in cell-free translation mixtures (13, 18). Additionally, all of the two-step mutants possess unstable 40S ribosomal subunits which dissociate into ribonucleoprotein core particles composed of only a few ribosomal proteins in high-ionic-strength ribosome wash buffers (13).

We isolated S14 cDNAs from a one-step *emtB* mutant (Em^r-2) and two independent double mutants derived from Em^r-2 (Em^r-2-2 and Em^r-2-3). All of the cDNAs were approximately the same size (Table 2), and all exhibited restriction endonuclease maps nearly identical to that illustrated in Fig. 1. The cDNAs listed in Table 2 were sequenced by the strategy depicted in Fig. 1. Except for a small number of single-base-pair differences (see Fig. 3 and Table 3), all of the cDNA sequences were identical.

```
                                        1>
-30        -20        -10        +1      10         20         30
ggtggagaag tctggagacg acgttcagaa ATGGCACCTC GCAAGGGAAA GGAAAAGAAG
                                  M  A  P   R  K  G  K   E  K  K

                                                  2>
         40         50         60         70         80         90
GAAGAACAGG TCATCAGCCT CGGACCTCAA GTGGCTGAAG GAGAGAATGT ATTTGGTGTC
 E  E  Q   V  I  S  L   G  P  Q   V  A  E   G  E  N  V   F  G  V

                                        3>       Sau3A
        100        110        120        130        140        150
TGCCACATTT TTGCATCTTT CAACGACACC TTTGTCCATG TTACTGATCT TTCTGGCAAG
 C  H  I   F  A  S  F   N  D  T   F  V  H   V  T  D  L   S  G  K

            HpaII<2,3        4>                     5> HinfI
        160        170        180        190        200        210
GAAACTATCT GCCGGGTGAC TGGTGGAATG AAGGTAAAGG CTGACAGAGA TGAGTCTTCT
 E  T  I   C  R  V  T   G  G  M   K  V  K   A  D  R  D   E  S  S

    6>       7> BglI          8>                      AluI
        220        230        240        250        260        270
CCTTATGCAG CCATGTTGGC TGCCCAGGAT GTTGCCCAGA GGTGCAAGGA GCTGGGCATC
 P  Y  A   A  M  L  A   A  Q  D   V  A  Q   R  C  K  E   L  G  I

                          AluI
        280        290        300        310        320        330
ACTGCCCTGC ATATCAAACT CCGAGCTACG GGAGGAAACA GGACCAAGAC CCCTGGACCT
 T  A  L   H  I  K  L   R  A  T   G  G  N   R  T  K  T   P  G  P

                    SstI/AluI            9><5,6,8
        340        350        360        370        380        390
GGAGCCCAGT CAGCCCTCAG AGCTCTTGCT CGTTCAGGGA TGAAGATTGG GCGGATTGAG
 G  A  Q   S  A  L  R   A  L  A   R  S  G   M  K  I  G   R  I  E

 10>
        400        410        420        430        440        450
GATGTCACCC CCATCCCCTC TGACAGCACC CGAAGGAAGG GTGGTCGTCG TGGTCGCCGT
 D  V  T   P  I  P  S   D  S  T   R  R  K   G  G  R  R   G  R  R

   <1,4,7,9                          PolyA-Addn         <10 AluI
        460        470        480        490        500        520
CTGTGAacag gacttctcaa attattttct gttaataaat tgctttgtat aagctaaaa..
 L
```

FIG. 2. Nucleotide sequence of pCS14-11 cDNA.This S14 cDNA is 550 base pairs long. Bases flanking the coding sequence are indicated in lowercase letters, and the S14 coding sequence is represented in uppercase letters. Landmark restriction endonuclease cleavage sites, a canonical polyadenylation site, and potential initiation (>) and termination (<) codons are underlined or indicated above the cDNA sequence or both. The polypeptide sequence encoded by the cDNA is represented by conventional single-letter amino acid abbreviations: A, alanine; R, arginine; N, asparagine; D, aspartate; C, cysteine; E, glutamate; Q, glutamine; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.

TABLE 1. Amino acid composition of Chinese hamster S14 protein[a]

Amino acid	Count	Mol %
Ala	15	9.9 (10.1)[b]
Arg	15	9.9 (10.7)
Asn	3	2.0
Asp	7	4.6
Cys	3	2.0
Gln	5	3.3
Glu	9	6.0
Gly	17	11.3 (11.7)
His	3	2.0 (2.7)
Ile	8	5.3 (4.4)
Leu	9	6.0 (7.3)
Lys	12	7.9 (7.8)
Met	4	2.6 (1.0)
Phe	4	2.6 (2.7)
Pro	7	4.6 (5.7)
Ser	9	6.0 (5.1)
Thr	10	6.6 (5.4)
Trp	0	0
Tyr	1	0.7 (1.7)
Val	10	6.6 (6.4)
Total	151	

[a] The amino acid composition of the CHO S14 protein was inferred from the long open reading frame in pCS14-11 cDNA (residues 1 through 453, Fig. 2).

[b] Data in parentheses are taken from the partial amino acid composition of rat liver ribosomal protein S14 determined by acid hydrolysis and chromatography (5).

Two single-base polymorphisms distinguished the CHO and CHL cell S14 cDNAs within protein-coding sequences. Correlated with the *Ava*I site polymorphism described above, residue 290 (see Fig. 2 for residue numbers) is a T in the CHO cell cDNAs and a C in the CHL cell cDNA. Thus, a proline codon in the cloned CHL S14 allele occupies the same position as a leucine codon in CHO S14 transcripts. Additionally, a silent third base difference in codon 127 distinguishes all CHO cDNAs from the CHL S14 sequence. Whereas GGA specifies Gly_{127} in the CHL S14 transcript, CHO S14 mRNAs use GGG (residues 379 to 381, Fig. 2). These might reflect either natural polymorphisms among the animals from which the CHO and CHL (V79) cell lines were isolated, differences that arose spontaneously in tissue culture, or artifacts of cloning the single CHL cDNA analyzed.

Mutations in two S14 codons appear to account for emetine resistance (*emtB*) and 40S ribosomal subunit instability. Both affected codons are within the extremely basic carboxyl sequence described above. In the Em^r-2 S14

TABLE 2. S14 cDNA clones used in these studies

cDNA	Source of mRNA	cDNA size (bp)[a]	Reference[b]
pCS14-1	CHL (V79), wild-type clone	495	18
pCS14-11	CHO, wild-type clone	568	t.r.
pCS14-8	CHO Em^r-2, low-level resistance	545	t.r.
pCS14-4	CHO Em^r-2-2, high-level resistance	515	t.r.
pCS14-9	CHO Em^r-2-3, high-level resistance	518	t.r.

[a] bp, Base pair.

[b] t.r., This report.

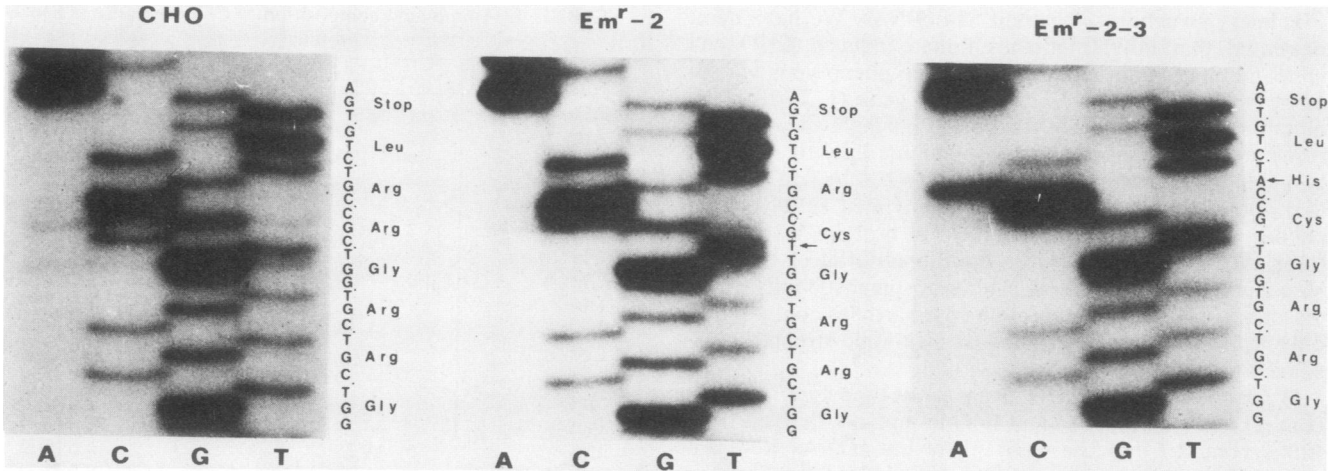

FIG. 3. Mutant S14 gene sequences responsible for emetine resistance of CHO cells. Comparable portions of the autoradiograms used to determine sequences of wild-type CHO (pCS14-11), Em^r-2 (pCS14-8), and Em^r-2-3 (pCS14-9) cDNAs are illustrated. In each case, the region of the autoradiogram shown encompasses the sequence encoding the S14 carboxyl-terminal heptapeptide. The DNA sequences read 5′ (bottom) to 3′ (top), and codon translations are to the right of each panel. Arrows denote base changes associated with each *emtB* mutation. A, Deoxyadenylate; C, deoxycytidylate; G, deoxyguanylate; T, deoxythymidylate.

cDNA, the C at position 445 was mutated to a T, changing Arg_{149} to Cys. Both second-step derivatives of Em^r-2 analyzed, although isolated from different mutagenized cultures (13), exhibited identical changes in the Arg_{150} codon. The G at residue 449 in the wild-type S14 cistron was mutated to A in Em^r-2-2 and Em^r-2-3, effecting His for Arg amino acid replacements. Autoradiograms of DNA sequencing gels that illustrate these differences are shown in Fig. 3.

Protein alterations that would result from these mutations are consistent with the electrophoretic properties of mutant S14 proteins. Em^r-2 S14 is slightly less basic than wild-type CHO S14 protein (1), reflecting a change in the carboxyl-terminal peptide from Gly-Arg_{149}-Arg-Leu to Gly-Cys_{149}-Arg-Leu. Em^r-2-2 and -2-3 S14 proteins are even less basic than Em^r-2 S14. This correlates with the change from Gly-Cys-Arg_{150}-Leu to Gly-Cys-His_{150}-Leu. That the second-step emetine resistance mutations reduce 40S ribosomal subunit stability indicates that Arg_{150} is important for interactions between protein S14 and other ribosomal subunit components, i.e., ribosomal RNAs or proteins or both. All of the single-base differences observed in CHL and CHO S14 genes, both spontaneous and ethyl methanesulfonate-induced mutations, are transitions. Two of the differences involved T-C polymorphisms, and two involved G-A polymorphisms (Table 3).

DISCUSSION

Chinese hamster cell S14 protein is one of the first mammalian ribosomal proteins whose complete primary sequence has been determined. At least 80 different genes make up the stringently regulated mammalian ribosomal protein gene family. Determinations of S14 and other ribosomal protein gene structures provide important first steps toward characterizing this important multi-gene family and mechanisms that regulate it. During the course of the research described above, primary sequences of two mouse ribosomal protein genes were published (6, 27).

The location of nucleic acid bases affected by emetine resistance mutations suggests an important structure-function relationship between the unusually basic S14 carboxyl peptide and the role of protein S14 in the protein biosynthetic function and architecture of the 40S ribosomal subunit. Our data indicate that mutation of the Chinese hamster S14 Arg_{149} codon results in low-level resistance to emetine and that mutation of the Arg_{150} codon, in addition to the Arg_{149} codon, produces high-level drug resistance and an unstable 40S ribosomal subunit. First- and second-step CHO *emtB* mutants displaying S14 proteins with electrophoretic migrations identical to those of our mutants have been described by others (3, 21). These are likely to involve nucleic acid substitutions similar or identical to the ones we describe. One *emtB* clone described in the literature appears to be a single-step mutant that elaborates an unstable 40S ribosomal subunit (26). It is tempting to speculate that the latter clone possesses a mutation affecting the Arg_{150} codon but not the Arg_{149} codon.

That Em^r-2-2 and Em^r-2-3 both experienced identical mutations leading to high-level emetine resistance, indistinguishable electrophoretic variants of the S14 protein, and unstable 40S ribosomal subunits may not be coincidence. The second-step mutants were isolated from separate mutagenized cultures of Em^r-2 (13) and therefore must represent independent genetic events. That both events affected the same codon identically probably indicates genetic constraints that derive from protein biosynthesis and ribosome assembly requirements.

Because eucaryotic ribosomal protein gene expression seems to be regulated, at least in part, post-transcriptionally (8, 10–12, 17, 19, 20, 22), we surveyed available rRNA and ribosomal protein sequences listed in the GenBank data base (BBN Laboratories, Cambridge, Mass.) for homologies to

TABLE 3. Point mutations and amino acid replacements affecting wild-type and emetine-resistant Chinese hamster cell S14 transcripts

cDNA residue	CHL (wild type)	CHO (wild type)	Em^r-2	Em^r-2-2 and Em^r-2-3
290	CCC(Pro_{97})	CTC(Leu_{97})	CTC(Leu_{97})	CTC(Leu_{97})
381	GGA(Gly_{127})	GGG(Gly_{127})	GGG(Gly_{127})	GGG(Gly_{127})
445	CGC(Arg_{149})	CGC(Arg_{149})	TGC(Cys_{149})	TGC(Cys_{149})
449	CGT(Arg_{150})	CGT(Arg_{150})	CGT(Arg_{150})	CAT(His_{150})

S14 cDNA. These included bacterial, amphibian, plant, murine, and rat genes. The only similarities discovered involved polypurine tracts such as that observed in S14 cDNA between residues −4 and +40 (Fig. 2). These were noted in a tobacco chloroplast S19 gene (25) and a rat rRNA spacer sequence (24). Whether these sequences participate in mechanisms that regulate the ribosomal gene family and how they might be involved in coordinating the expression of ribosomal protein and rRNA genes is not clear. A greater sample of mammalian ribosomal protein gene structures is necessary to discern sequences that govern this gene family.

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LITERATURE CITED

1. **Boersma, D., S. M. McGill, J. Mollenkamp, and D. J. Roufa.** 1979. Emetine resistance in Chinese hamster cells. Analysis of ribosomal proteins prepared from mutant cells. J. Biol. Chem. **254**:559–567.
2. **Boersma, D., S. M. McGill, J. W. Mollenkamp, and D. J. Roufa.** 1979. Emetine resistance in Chinese hamster cells is linked genetically with an altered 40S ribosomal subunit protein S20. Proc. Natl. Acad. Sci. U.S.A. **76**:415–419.
3. **Chang, S., and J. J. Wasmuth.** 1983. Construction and characteristics of Chinese hamster cell EmtA EmtB double mutants. Mol. Cell. Biol. **3**:761–772.
4. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
5. **Collatz, E., N. Ulbrich, K. Tsurugi, H. N. Lightfoot, W. MacKinlay, A. Lin, and I. G. Wool.** 1977. Isolation of eukaryotic ribosomal proteins. Purification and characterization of the 40S ribosomal subunit proteins. J. Biol. Chem. **252**:9071–9080.
6. **Dudov, K. P., and R. P. Perry.** 1984. The gene family encoding the mouse ribosomal protein L32 contains a uniquely expressed intron-containing gene and an unmutated processed gene. Cell **37**:457–468.
7. **Elder, J. H., R. A. Pickett, J. Hampton, and R. A. Lerner.** 1977. Radioiodination of proteins in single polyacrylamide gel slices. J. Biol. Chem. **252**:6510–6515.
8. **Geyer, P. K., O. Meyuhas, R. P. Perry, and L. F. Johnson.** 1982. Regulation of ribosomal protein mRNA content and translation in growth-stimulated mouse fibroblasts. Mol. Cell. Biol. **2**:685–693.
9. **Gubler, U., and B. J. Hoffman.** 1983. A simple and very efficient method for generating cDNA libraries. Gene **25**:263–269.
10. **Ignotz, G. G., S. Hokari, R. M. De Philip, K. Tsukada, and I. Lieberman.** 1981. Lodish model and regulation of ribosomal protein synthesis by insulin-deficient chick embryo fibroblasts. Biochemistry **20**:2550–2558.
11. **Kief, D. R., and J. R. Warner.** 1981. Coordinate control of synthesis of ribosomal ribonucleic acid and ribosomal proteins during nutritional shift-up in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:1007–1015.
12. **Krauter, K. S., R. Soeiro, and B. Nadal-Ginard.** 1980. Uncoordinate regulation of ribosomal RNA and ribosomal protein synthesis during 16e9 myoblast differentiation. J. Mol. Biol. **142**:145–159.
13. **Madjar, J.-J., M. Frahm, S. McGill, and D. J. Roufa.** 1983. Ribosomal protein S14 is altered by two-step emetine resistance mutations in Chinese hamster cells. Mol. Cell Biol. **3**:190–197.
14. **Madjar, J.-J., K. N. Smith, M. Frahm, and D. J. Roufa.** 1982. Emetine resistance in Chinese hamster ovary cells is associated with an altered ribosomal protein S14 mRNA. Proc. Natl. Acad. Sci. U.S.A. **79**:1003–1007.
15. **Maxam, A., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
16. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
17. **Nabeshima, Y.-I., and K. Ogata.** 1980. Stimulation of the synthesis of ribosomal proteins in regenerating rat liver with special reference to the increase in the amounts of effective mRNAs for ribosomal proteins. Eur. J. Biochem. **107**:323–329.
18. **Nakamichi, N., D. D. Rhoads, and D. J. Roufa.** 1983. The Chinese hamster cell emetine resistance gene: analysis of cDNA and genomic sequences encoding ribosomal protein S14. J. Biol. Chem. **258**:13236–13242.
19. **Pearson, N. J., H. M. Fried, and J. R. Warner.** 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell **29**:347–355.
20. **Ramagopal, S., and H. L. Ennis.** 1981. Regulation of synthesis of cell-specific ribosomal proteins during differentiation of *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. U.S.A. **78**:3083–3087.
21. **Reichenbecher, V. E., and C. T. Caskey.** 1979. Emetine-resistant Chinese hamster cells. J. Biol. Chem. **254**:6207–6210.
22. **Santon, J. B., and M. Pellegrini.** 1980. Expression of ribosomal proteins during Drosophila early development. Proc. Natl. Acad. Sci. U.S.A. **77**:5649–5653.
23. **Smith, A. J. H.** 1980. DNA sequence analysis by primed synthesis. Methods Enzymol. **65**:560–580.
24. **Subrahmanyam, C. S., B. Cassidy, H. Busch, and L. I. Rothblum.** 1982. Nucleotide sequence of the region between the 18S rRNA sequence and the 28S rRNA sequence of rat ribosomal DNA. Nucleic Acids Res. **10**:3667–3680.
25. **Sugita, M., and M. Sugiura.** 1983. A putative gene of tobacco chloroplast coding for ribosomal protein similar to *E. coli* ribosomal protein S19. Nucleic Acids Res. **11**:1913–1918.
26. **Wejksnora, P. J., and J. R. Warner.** 1979. Hybrid mammalian cells assemble hybrid ribosomes. Proc. Natl. Acad. Sci. U.S.A. **76**:5554–5558.
27. **Wiedemann, L. M., and R. P. Perry.** 1984. Characterization of the expressed gene and several processed pseudogenes for the mouse ribosomal protein L30 gene family. Mol. Cell. Biol. **4**:2518–2528.