MOLECULAR AND CELLULAR BIOLOGY, Oct. 1987, p. 3767–3774
0270-7306/87/103767-08$02.00/0

Vol. 7, No. 10

A Cloned Human Ribosomal Protein Gene Functions in Rodent Cells†

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Received 18 May 1987/Accepted 16 July 1987

Cloned fragments of human ribosomal protein S14 DNA (RPS14) were transfected into cultured Chinese hamster (CHO) cells. Transient expression assays indicated that DNA with as little as 31 base pairs of upstream flanking sequence was transcribed into a polyadenylated, 650-base mRNA that is largely bound to the polyribosomes. In these respects the exogenous human S14 message appeared to function normally in CHO cells. Interestingly, transcription of human RPS14 did not require the TATA sequence located 26 base pairs upstream of exon 1. Stably transformed clones were selected from cultures of emetine-resistant CHO cells (Em^r-2) after transfection with pSV2Neo-human RPS14 constructs. Human RPS14 complemented the mutationally based drug resistance of the Chinese hamster cells, demonstrating that the cloned human ribosomal protein gene is functional in rodent cells. Analysis of transformed cells with different amounts of integrated RPS14 indicated that human S14 mRNA levels are not tightly regulated by CHO cells. In contrast, the steady-state S14 level fluctuated only slightly, if at all, in transformed clones whose S14 message contents differed by more than 30-fold. These data support the conclusion that expression of human RPS14 is regulated, at least partially, posttranscriptionally.

Ribosomal components are encoded by a complex family of genes in eucaryotic and procaryotic organisms. Because approximately equal amounts of ribosomal proteins (r-proteins) and rRNAs are synthesized under various growth conditions (11, 13, 19, 21, 28, 30, 36, 40, 45), ribosomal genes appear to be controlled by stringent biochemical and genetic mechanisms. Expression of r-protein genes is governed by a combination of transcriptional, translational, and posttranslational mechanisms in bacteria and lower eucaryotes (12, 15, 20, 29, 33, 35, 39, 43, 44, 47–49). Comparable information about eucaryotic, especially mammalian, r-protein genes is sparse owing to a lack of high-resolution genetic systems.

Only one of the mammalian r-protein genes so far isolated is amenable to genetic analysis in tissue culture. Recessive mutations (*EmtB*) affecting the CHO cell r-protein S14 gene (RPS14) result in resistance to emetine, an alkaloid inhibitor of protein biosynthesis (2, 3, 16, 17, 24). *EmtB* S14 protein is slightly more acidic than wild-type S14 (2), and ribosomes containing mutant S14 are relatively unstable (23, 45). RPS14 genomic and cDNA sequences have been cloned from Chinese hamster and human cells, and their complete nucleic acid sequences are known (32, 37, 38). Human RPS14 consists of five exons and four introns spanning 6 kilobase pairs (kbp) of chromosome 5q DNA (37). It contains a TATA box 28 bp upstream of exon 1. In this regard RPS14 differs from the few other well-characterized mammalian (mouse) r-protein genes, which all lack TATA sequences (9, 42, 46). Because human 5q23-q33 chromosome fragments rescue drug resistance in interspecific CHO *EmtB* × human cell hybrids (8, 31), assignment of the RPS14 genomic DNA clones to a transcriptionally active locus rests on compelling somatic genetic as well as molecular data.

We transfected human RPS14 fragments into *EmtB* CHO cells and assayed for functional human S14 mRNA and protein. Our data demonstrated that the cloned DNAs encode a complete and functional r-protein gene. Disrupting the TATA sequence immediately 5′ of exon 1 (37) did not preclude transcription of S14 mRNA, which was accurately spliced, polyadenylated, and loaded onto polyribosomes in Chinese hamster cells. Indeed, synthesis of S14 message required only upstream DNA sequence within 31 bp of RPS14 exon 1. Analysis of CHO cells stably transformed with pSV2Neo-RPS14 constructs suggested that human RPS14 is transcribed constitutively in hamster cells and that steady-state levels of S14 protein are regulated posttranscriptionally.

MATERIALS AND METHODS

Materials. Human and Chinese hamster RPS14 genomic and cDNA clones used in these studies have been described previously (7, 32, 37, 38). The genomic clone, HGS14-1, consists of 12.5 kbp of human placental DNA including the entire RPS14 gene in the lambda Charon 28 vector (37). pCS14-1 is an approximately full-length cDNA (520 bp) derived from size-fractionated Chinese hamster cell polyadenylated mRNA (32). pCS14-12 is a similar clone (546 bp) isolated from human (HeLa cell) message (37). Both S14 cDNAs encode identical 151-amino acid polypeptides (7). cDNAs specifying two Chinese hamster r-protein messages, S17 and L36a, have been described before (32). *EmtB* Chinese hamster ovary (CHO) cells, Em^r-2 (2, 3, 23), were used as recipients in transfection experiments.

Construction of RPS14 subclones. A partial restriction site map of HGS14-1 is depicted at the top of Fig. 1. Translation of the gene initiates near the beginning of RPS14 exon 2 and proceeds from left to right as illustrated (Fig. 1, arrow). Digestion of HGS14-1 with *Hin*dIII and limiting amounts of *Eco*RI yielded DNA fragments with a common downstream end (the *Hin*dIII site 3′ to exon 5) and various *Eco*RI cleavage sites as upstream termini. Two fragments, pS14 (−4000) and pS14(−700), were used in the experiments reported. To facilitate handling, their staggered ends were

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† Contribution no. 87-427-J from the Kansas Agricultural Experiment Station.

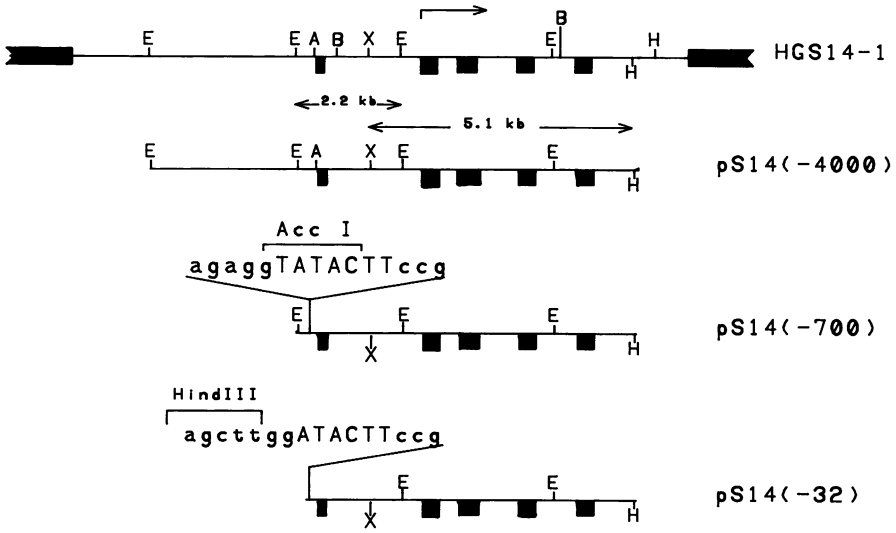

FIG. 1. Construction of expressible human RPS14 subclones. A 12.5-kbp human placental RPS14 fragment cloned in the lambda vector Charon 28 (HGS14-1) (37) is illustrated at the top of the figure. Five exons are indicated by boxes. S14 protein-coding sequence initiates near the start of exon 2 (arrow). Landmark restriction endonuclease cleavage sites are indicated: E, *Eco*RI; A, *Acc*I; B, *Bam*HI; H, *Hin*dIII; and X, *Xho*I. Construction of the subclones pS14(−4000), pS14(−700), and pS14(−32) is described in the text.

filled with *Escherichia coli* DNA polymerase I, modified with *Hin*dIII linkers (New England BioLabs, Inc., Beverly, Mass.), and subcloned in the plasmid vector pUC13 (Fig. 1). The resulting clones include the entire RPS14 coding sequence and, as their names indicate, approximately 4.0 and 0.7 kbp of upstream flanking DNA, respectively.

To test the role of the upstream flanking TATA sequence in promoting RPS14 transcription, we engineered a truncated clone in which the TATA box is disrupted by a linker-insertion mutation. The 2.2-kbp *Eco*RI DNA fragment containing all of exon 1 and most of intron 1 (Fig. 1) was purified from pS14(−700) and cleaved at the *Acc*I site within the TATA sequence. The cleavage products were modified with *Hin*dIII linkers and resolved by electrophoresis. The fragment containing exon 1 then was ligated to the 3′ portion of RPS14 at the *Xho*I site within intron 1 to construct pS14(−32) (Fig. 1). The structure of this clone was confirmed by a detailed restriction endonuclease map and by determining its nucleic acid sequence upstream of exon 1.

For stable transformation assays, the human DNA in pS14(−4000) was subcloned into the gap-filled *Bam*HI site of pSV2Neo (41), yielding the constructs pSVNS14-1 and -4 (see Fig. 4).

DNA transfection, transient expression, and stable transformation. Monolayer cultures of CHO Em^r-2 cells (approximately 10^6 cells per 10-cm dish) were transfected with cloned RPS14 fragments (300 ng) in the presence of Polybrene (10 μg/ml; Aldrich Chemical Co., Inc., Milwaukee, Wis.) as described previously (6). After 6 h at 37°C, cultures were rinsed with medium containing 30% (vol/vol) dimethyl sulfoxide and refed with growth medium. Twenty-four hours later, the neomycin analog G418 (GIBCO Laboratories, Inc., Grand Island, N.Y.) was added to selection medium (500 μg/ml). Cells were refed on day 7, and by day 14 approximately 40 G418-resistant colonies per μg of transforming DNA were visible. Representative colonies were purified in medium containing 250 μg of G418 per ml (14).

In transient expression experiments, Em^r-2 cells were treated with 10 to 20 μg of a cloned DNA fragment by the Polybrene protocol outlined above. Twenty-four hours later cells were scraped from culture dishes and lysed in buffer containing 0.5% Nonidet P−40. RNA was purified from cytoplasmic subcellular fractions (18, 24).

Expression of human RPS14 in CHO cells. S14 message was detected among cytoplasmic RNAs by stringent filter blot nucleic acid hybridization procedures (32). Two approaches were followed: cytoplasmic RNAs (5 to 20 μg) were applied to GeneScreen Plus (New England Nuclear Corp., Boston, Mass.) slot blots; and r-protein transcripts were assayed by nucleic acid hybridization with nick-translated human and hamster [^{32}P]cDNAs (1×10^8 to 2×10^8 cpm/μg) as probes (7, 32). Final posthybridization washes were done in 0.5× SSC (1× SSC is 0.15 M NaCl, plus 0.015 M sodium citrate, pH 7.0) at 65°C. Autoradiographic bands were amenable to accurate quantitation by densitometry. Alternatively, formamide-denatured RNAs were electrophoresed through 1.2% (wt/vol) agarose in buffer containing 2.2 M formaldehyde. Gels were blotted to GeneScreen Plus filters and analyzed by nucleic acid hybridization. The latter procedure ensured that mRNAs identified comigrated electrophoretically with authentic S14 message (32).

Human and Chinese hamster S14 mRNAs also were resolved by an S1 nuclease protection assay (1). Species-specific sense-strand cDNA probes were subcloned into the single-stranded bacteriophage vector M13mp11 (27). The human S1 probe extends from the 5′ end of pCS14-12 (a *Pst*I site) to an *Alu*I site at position 311 (37) and includes sequence encoded by RPS14 exons 1 to 3. The Chinese hamster probe extends from the 5′ terminus of pCS14-1 (also a *Pst*I site) to an *Sst*I site 345 bases downstream (32). It contains sequences from exons 2 to 4. Probes were radiolabeled (1×10^8 to 2×10^8 cpm/μg) with the M13mp11 subclones as the templates, M13 sequencing primer (P-L Biochemicals, Inc., Milwaukee, Wis.), [α-^{32}P]dCTP (New England Nuclear Corp.), and *E. coli* DNA polymerase I Klenow fragment (United States Biochemical Corp., Cleveland) (5).

S1 nuclease protection assays were performed as follows. RNA samples (10 μg) were mixed with 10^6 cpm of S1 probe

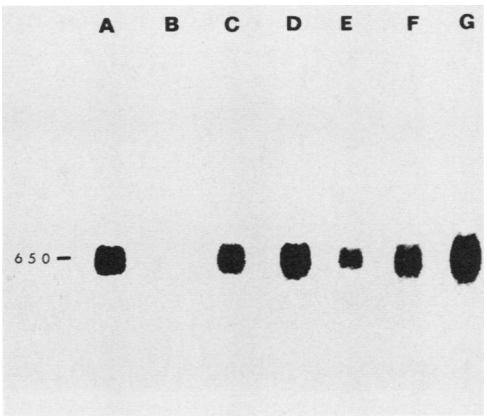

FIG. 2. Human S14 message is transcribed by Chinese hamster cells after RPS14 transfection. Cytoplasmic RNAs (10 μg) from transfected and control cell lines were electrophoresed through a denaturing 1.2% agarose gel and blotted to a filter membrane as described in Materials and Methods. The filter was analyzed by nucleic acid hybridization (see text) with human S14 [^{32}P]cDNA as the probe. Lanes: A, HeLa cell RNA; B, CHO cell RNA; C to G, RNA from Em^r-2 cells transiently expressing pS14(−4000), pS14(−700), pS14(−32), pSVNS14-1, and pSVNS14-4 subclones, respectively. Number shows 650 bases.

in 30 μl of annealing buffer (80% formamide, 0.4 M NaCl, 1 mM EDTA, 40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], pH 6.4), heat denatured for 10 min at 90°C, and allowed to hybridize for 6 h at 50°C. Mixtures were diluted with 300 μl of ice-cold digestion buffer (50 mM NaCl, 50 mM sodium acetate [pH 4.6], 4.5 mM $ZnSO_4$, 20 μg of sonicated, single-stranded herring DNA per ml) and then treated with 900 U of S1 nuclease (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). After 120 min at 30°C, 10 μg of carrier tRNA was added. S1 nuclease-resistant fragments were analyzed by electrophoresis through denaturing 6% polyacrylamide gels (1). Molecular sizes were estimated using end-labeled DNA standards. For quantitative comparison of hamster versus human RPS14 transcripts, transfected cell RNAs were annealed with an equimolar mixture of hamster and human [^{32}P]cDNA probes. Amounts of the two messages then were calculated from densitometry scans of the autoradiograms, using appropriate corrections for relative probe sizes and base compositions.

G418-resistant colonies were tested for sensitivity to emetine by relative plating efficiencies in G418 media (500 μg/ml) with and without emetine hydrochloride (10^{-7} M). Steady-state levels of S14 protein in transformed and control cells were monitored by two-dimensional polyacrylamide gel electrophoresis (see legend to Fig. 8).

RESULTS

Transient expression of human RPS14 in Chinese hamster cells. To determine whether the cloned human RPS14 fragments include a complete r-protein transcriptional unit, to recognize the minimum 5′-flanking sequence required for S14 mRNA transcription, and to establish whether the upstream TATA sequence is required for S14 transcription, we performed the transient expression experiments summarized in Fig. 2. Preliminary data indicated that CHO cells transcribe a relatively constant level of human S14 message between 12 and 60 h posttransfection. Thus, we assayed human S14 mRNA in CHO cells 24 h after transfection with the RPS14 DNA constructs (Fig. 2).

All the RPS14 fragments examined directed transcription of human RPS14 mRNA. Nucleic acid hybridization conditions used to analyze the filter blot illustrated in Fig. 2 were stringent enough to eliminate most cross-reaction with resident CHO S14 mRNA (Fig. 2, lane A versus lane B). Regardless of the length of upstream human sequence contained by template DNAs (32 bp to 4 kbp) or of the cloning vectors used (pUC13 versus pSV2Neo) or of the orientations of the human DNAs within the cloning vectors (pSVNS14-1 versus pSVNS14-4), levels of human S14 mRNA elaborated were not significantly different. Slight variations in band intensities (lanes E and G versus lanes C, D, and F) were not reproducible, apparently reflecting individual transfection efficiencies. Therefore, the minimum upstream DNA sequence required to promote transcription of human RPS14 resides within 31 bp of exon 1. More important and surprising in view of the primary structure of the gene (37), disruption of the TATA sequence in pS14(−32) did not prevent transient expression of the gene, nor did it significantly alter the size of the mRNA transcribed (Fig. 2, lane E versus lanes C and D). Further, as pSV2Neo did not promote reproducibly higher levels of expression than pUC13 (lanes F and G versus lanes C to E), RPS14 transcription was not markedly affected by the powerful simian virus 40 early-gene enhancer sequence contained in pSV2Neo.

Virtually all the human S14 mRNA elaborated by transformed cells binds to oligo(dT)-cellulose and fractionates with the polyribosomes of the cells, suggesting that it is both polyadenylated and available for translation. Human S14 message is the same size (650 bases) in transiently expressing hamster cells as in HeLa cells (Fig. 2, lanes C to G versus lane A). S1 nuclease protection experiments confirmed that pre-mRNA sequences derived from exons 1 to 3 were spliced exactly as they are in normal S14 message (see Fig. 7). In sum, mechanisms required for processing human RPS14 pre-mRNA and for loading it onto cytoplasmic polyribosomes appear to be operative in Chinese hamster cells.

***EmtB* Chinese hamster cells stably transformed with human RPS14.** *EmtB* mutant CHO cells were used to determine whether the human S14 mRNA was translated accurately by Chinese hamster cells and whether the human protein was transported into hamster cell nucleoli and assembled into translationally active, chimeric 40S ribosomal subunits. Rescue of the mutationally based *EmtB* drug resistance phenotype in stably transformed cells would confirm that human RPS14 functions in Chinese hamster cells. In addition, a collection of stably transformed somatic Chinese hamster cells would permit us to investigate effects of r-protein gene number and arrangement on regulated expression of human RPS14.

Em^r-2 cells were transformed with pSVNS14-1 and -4. Of 53 G418-resistant clones isolated, 23 (43%) proved sensitive to emetine. In these, human RPS14 phenotypically complemented the resident CHO *EmtB* gene, regardless of the orientation of the cloned DNA within the pSV2Neo vector (see Fig. 4).

To ascertain the amount and arrangement of integrated RPS14 sequences in transformed cells, we analyzed their DNAs by stringent Southern filter blots by using human S14 cDNA as the hybridization probe. In the blot illustrated (Fig. 3), DNAs were digested with *Bam*HI. Intact human RPS14 yielded a 4.8-kbp *Bam*HI fragment (Fig. 4). Other clone-specific bands containing human-vector, human-hamster,

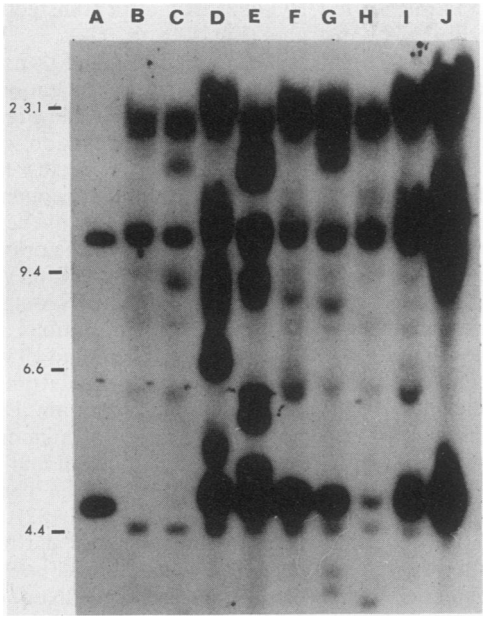

FIG. 3. Human RPS14 DNA sequence within stably transformed CHO cell chromosomes. DNAs (10 μg) from transformed Em^r-2 cell lines (lanes C to J) were digested with *Bam*HI, electrophoresed through agarose, and blotted to a filter membrane. The filter was probed with nick-translated human S14 [^{32}P]cDNA and assayed by autoradiography. Lane A contained 20 pg of pSVNS14-1 DNA (see Fig. 4); lane B contained wild-type CHO DNA; and lane C contained DNA from an emetine-resistant transformant, S14-12a. Lanes D to J contained DNAs from emetine-sensitive transformed lines: D, S14-2a; E, S14-4a; F, S14-10a; G, S14-10b; H, S14-12c; I, S14-14a; and J, S14-18a. Numbers on left are in kilobase pairs.

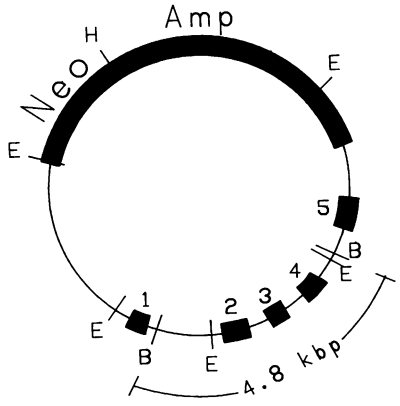

FIG. 4. pSV2Neo-RPS14 constructs. The 10.5-kbp *Bam*HI fragment of human RPS14 (Fig. 1) was inserted into pSV2Neo. In pSVNS14-1, human RPS14 5′-flanking sequences are proximal to the G418 resistance gene of the vector. Human DNA is represented by a thin line; pSV2Neo DNA by a thick line; RPS14 exons by numbered boxes; and the G418 and ampicillin resistance genes of the vector by Neo and Amp, respectively. Restriction endonuclease sites used to determine human DNA orientation within the construct are indicated (E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI), as is the 4.8-kbp *Bam*HI fragment diagnostic of the intact RPS14 cloned. pSVNS14-4 carries the human RPS14 fragment in an orientation opposite to the one illustrated.

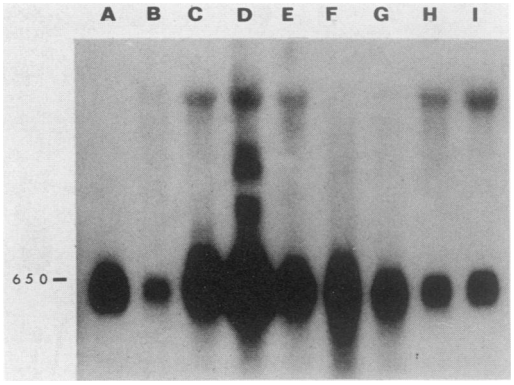

FIG. 5. Stably transformed CHO cells elaborate normal-size human S14 message. Cytoplasmic RNAs (10 μg) were purified from HeLa cells (lane A); CHO Em^r-2 cells (lane B); five emetine-sensitive transformed clones, S14-2a, -4a, -10a, -10b, and -18a (lanes C to G, respectively), an emetine-resistant transformant, S14-11a (lane H); and cells transformed by the pSV2Neo vector (lane I). These were electrophoresed through a denaturing agarose gel, blotted to a filter membrane, and analyzed under moderately stringent hybridization conditions with a nick-translated human S14 cDNA probe. Number on left shows 650 bases.

and perhaps, human-vector-hamster hybrid DNA sequences also were anticipated. Note that the diagnostic 4.8-kbp *Bam*HI fragment was observed in pSVNS14-1 DNA (Fig. 3, lane A) and in all DNAs from emetine-sensitive transformed CHO cell lines (Fig. 3, lanes D to J), but not in CHO cell DNA (lane B). The intensity of this band differed from clone to clone, suggesting that each subline harbors a characteristic amount of human RPS14 coding sequence. In addition, the DNAs all contain unique human S14 bands, apparently reflecting clone-specific DNA rearrangements and chromosomal sites of integration. It is important to emphasize, however, that Southern blots (Fig. 3) provide only estimates of the amount and organization of human RPS14 within the chromosomes of transformed cells. They do not distinguish between transcriptionally active and rearranged or otherwise inactive human DNA sequences.

Electrophoretic analyses indicated that most of the emetine-sensitive cell lines elaborated a minor, 1000-base S14 RNA species as well as the 650-base mature r-protein message (Fig. 5). Both RNAs have been described before (32). Clone S14-4a, which contains several, apparently rearranged, human RPS14 sequences (Fig. 3, lane E), displayed a complex pattern of S14 RNAs (Fig. 5, lane D). These unusual transcripts probably are products of nonfunctional fused genes, or they are normally transient, pre-mRNA intermediates stabilized by one or more rearrangements involving integrated DNA sequences.

The intensity of S14 bands in transformed cell RNA blots suggested that steady-state S14 mRNA levels varied among the clones. To examine this point more critically, we slot blotted RNAs to a filter membrane. Blots were assayed by hybridization with an equimolar mixture of human and Chinese hamster S14 cDNA probes (see Materials and Methods), and resulting autoradiograms were scanned with a densitometer (Fig. 6). Thus, the scan in Fig. 6A reflects both human and hamster transcripts and indicates that clonal S14 mRNA levels varied over approximately a 35-fold range. As in the Northern filter blots (RNA blots) (Fig. 5), S14-4a (Fig. 6, slot 3), S14-12e (slot 8), and S14-21c (slot 14) displayed the highest amounts of S14 RNA. To investigate the effect of

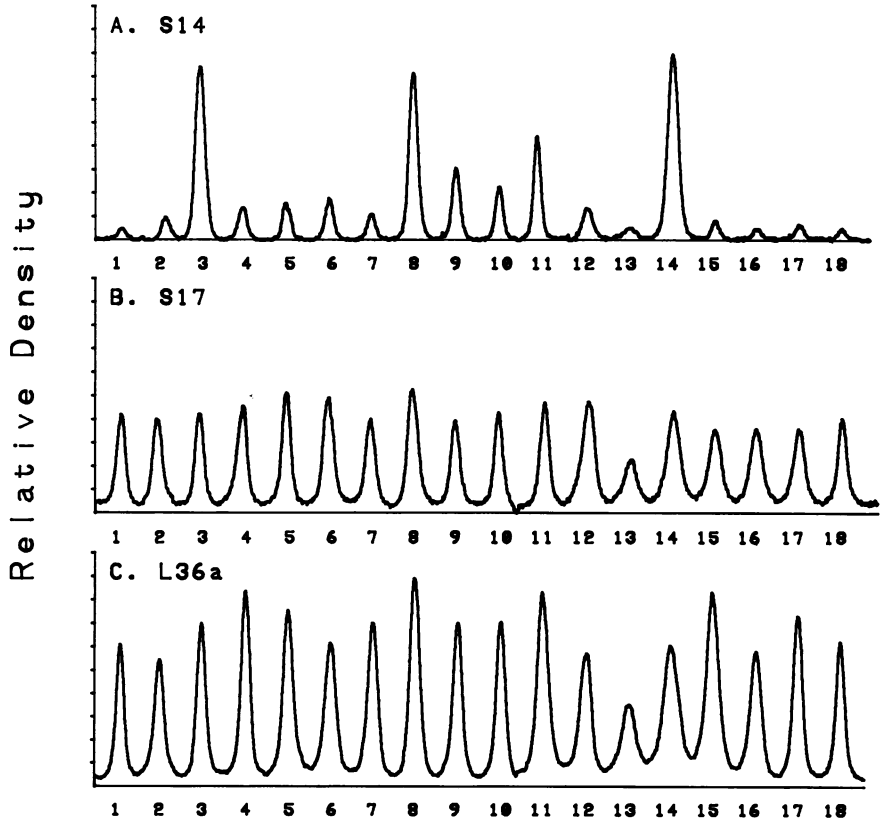

FIG. 6. r-Protein message levels in stably transformed CHO cells. Cytoplasmic RNAs (10 μg) from transformed and control cell lines were slot blotted to a filter membrane and analyzed by nucleic acid hybridization with an equimolar mixture of human and CHO S14 cDNAs (A), CHO S17 cDNA (B), and CHO L36a cDNA (C) ^{32}P-labeled hybridization probes. Slot 1 contained CHO Em^r-2 RNA. Slots 2 to 14 were loaded with RNA from the emetine-sensitive clones S14-2a, -4a, -5a, -10a, -10b, -12c, -12e, -13a, -14a, -16a, -16b, -18a, and -21c, respectively. Slots 15 to 17 contained RNA from the emetine-resistant clones S14-5b, -11a, and -12a, respectively. Slot 18 included RNA from a CHO cell transformed by the pSV2Neo vector as a control.

multiple active RPS14 genes on expression of other Chinese hamster cell r-protein genes, we removed radioactive S14 probe from the filter membrane, and the blot was reanalyzed with radioactive Chinese hamster RPS17 (Fig. 6B) and RPL36a (Fig. 6C) cDNA probes. In sharp contrast to the consistent, clone-specific S14 RNA levels observed (Fig. 6A), only slight (and nonreproducible) differences among clonal S17 and L36a message levels were detected (Fig. 6B and C).

The composition (CHO versus human) of S14 mRNA in transformed cells was assessed by S1 nuclease protection. Human S14 mRNA (Fig. 7, lane A) protected a heterogeneous set of human cDNA fragments 260 to 280 nucleotides in length. Hamster mRNA protected three CHO probe fragments 300 to 315 bases long (Fig. 7, lane D). Neither probe was protected by heterologous mRNAs under the assay conditions used (lanes B and C). Lane M contained RNA from an emetine-resistant transformed clone (S14-12a), and lane N contained RNA from CHO cells transformed by the pSV2Neo vector. As expected, these two samples included only CHO S14 mRNA. Ratios of human to hamster mRNAs varied considerably within the transformed cells (lanes F to L). The greatest amount of human message was transcribed by S14-4a (lane G) and S14-6b (lane I), two clones that harbor multiple integrated S14 sequences (Fig. 3).

In general, data in Fig. 3 and 5 to 7 support three conclusions. (i) Steady-state levels of S14 mRNA are highest in transformed clones that contain large amounts of integrated human RPS14 sequence. (ii) Elevated levels of S14 message derive primarily from integrated human, not resident CHO, RPS14. (iii) Enhanced RPS14 transcription did not affect steady-state levels of at least two other CHO r-protein messages, S17 and L36a.

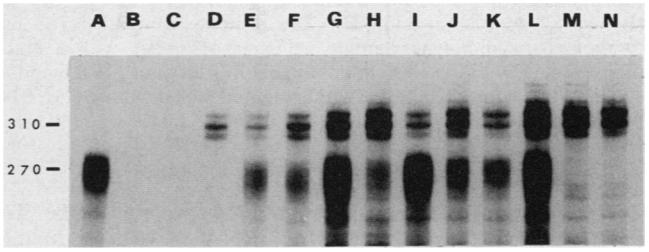

FIG. 7. Stably transformed CHO cells elaborate both human and hamster S14 mRNAs. Cytoplasmic RNAs (20 μg) from HeLa cells (lanes A and C), CHO Em^r-2 cells (lanes B and D), and a mixture of HeLa and Em^r-2 RNAs (10 μg each, lane E) were assayed by S1 nuclease protection with human (lanes A and B) and CHO (lanes C and D) S14 [^{32}P]cDNA probes. Lanes E to N were analyzed with an equimolar mixture of the two [^{32}P]cDNA probes (see text). Lanes F to L were loaded with RNA from emetine-sensitive transformed cells: S14-2a, -4a, -5a, -6b, -10a, -18a, and -21c, respectively. Lane M contained RNA from an emetine-resistant transformed clone (S14-12a), and lane N contained RNA from a clone transformed by the pSV2Neo vector. Numbers on left are in nucleotides.

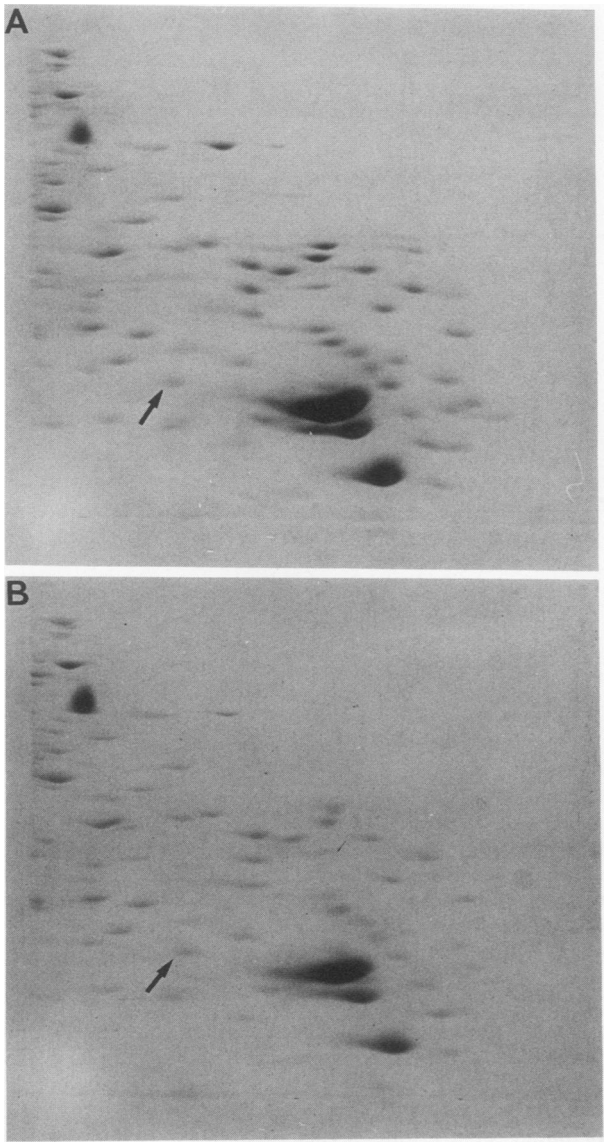

FIG. 8. r-Proteins in normal and transformed CHO cells. Total cell proteins were extracted from CHO Em^r-2 cells (A) and a transformed clone that overproduces human S14 message, S14-4a (B), in 5.5 M acetic acid (23). The proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis with a first-dimension (1D) buffer system containing 8 M urea at pH 8.6 and a second-dimension (2D) buffer with sodium dodecyl sulfate (23). S14 protein is indicated by an arrow.

Posttranscriptional regulation of S14 protein levels. To determine whether all the S14 mRNA in overproducing hamster cell transformants is translated into protein (leading to accumulation of free S14 protein) or whether posttranscriptional mechanisms affect final r-protein levels, we analyzed steady-state amounts of S14 protein in normal and stably transformed CHO cells. Acid-soluble proteins were extracted from Em^r-2 and S14-4a, a clone that transcribes an extremely large amount of human RPS14 message (Fig. 5 to 7). These were analyzed by two-dimensional polyacrylamide gel electrophoresis. The r-protein patterns observed are illustrated in Fig. 8. S14 protein (arrow) was identified from its mobility in multiple two-dimensional gel systems (23, 25, 26). The four intense spots to the right of and above S14 are the major histone proteins. This semi-quantitative assay indicates that the two cell lines elaborate comparable amounts (within twofold) of S14, despite the approximately 35-fold difference in their steady-state S14 mRNA levels. Additionally, amounts of S14 protein relative to other nearby r-proteins are approximately the same in S14-4a whole-cell extracts (Fig. 8B) and in extracts of highly purified CHO cell ribosomal subunits (2, 24, 32). This suggests that, despite their abundance of S14 mRNA, transformed cells do not contain a significant pool of soluble S14 protein. Because virtually all the S14 mRNA elaborated by clone 4a is polyadenylated and associated with the polyribosomes of the cells, either excess S14 mRNA was not translated efficiently or most of the S14 protein synthesized was degraded rapidly. Whatever the case, the data suggest that intracellular levels of mammalian r-protein S14 are regulated posttranscriptionally.

DISCUSSION

Transient expression verified that the human RPS14 fragments cloned contain a complete, transcriptionally active r-protein gene. Analysis of truncated clones indicated further that upstream DNA sequences necessary for mRNA biosynthesis reside within 31 bp of RPS14 exon 1. In these respects, human RPS14 appears similar to mouse RPL32 and RPS16 (10). Unlike other mammalian r-protein genes whose structures are known (9, 42, 46), human RPS14 contains a TATA sequence motif (TATACTT) (4) 26 bp 5′ of exon 1 (37). Much to our surprise, transcription of a truncated RPS14 fragment that terminates within the TATA motif revealed that it is not absolutely required for RPS14 transcription. Whether or not the TATA box affects the RPS14 promoter is unclear at this time. There is a small but real possibility that a DNA sequence only coincidently resembling the TATA motif occupies a position 26 bp upstream of RPS14 by chance alone.

One striking resemblance among the few r-protein genes studied by transient expression is the limited amount of 5′-flanking sequence required for their transcription. The promoter of murine RPL32 extends less than 36 bp upstream of exon 1 (10). Proximal upstream flanking sequences in human RPS14, murine RPL32 (9), RPL30 (46), RPS16 (42), *Xenopus laevis* RPL1 (22), and *Drosophila melanogaster* RP49 (34) include short inverted DNA sequence motifs 3 to 7 bp 5′ of their mRNA initiation sites (Fig. 9). Most of the inverted motifs are imperfect, and all surround asymmetric 4 to 7 bp centers. Perhaps these sequence elements are important determinants of eucaryotic r-protein gene promoters. One fact is known: cells that harbor a mouse RPL32 minigene with an upstream deletion extending into the motif (to position −13, * in Fig. 9) do not initiate transcription accurately (10).

S14 DNA fragments rescue emetine resistance in stably transformed CHO Em^r-2 cells, confirming that the cloned DNAs encode the functional human RPS14 locus and that heterologous S14 protein is transported, processed, and assembled into active ribosomal subunits in Chinese hamster cells. Levels of human S14 message associated with polyribosomes were highest in cells that harbored multiple integrated copies of human RPS14 DNA. However, clone S14-4a, which elaborated 30- to 40-fold more S14 message than control cells, did not accumulate detectable quantities of free S14 protein. This implies that the intracellular pool of S14 protein is regulated, at least partially, posttranscription.

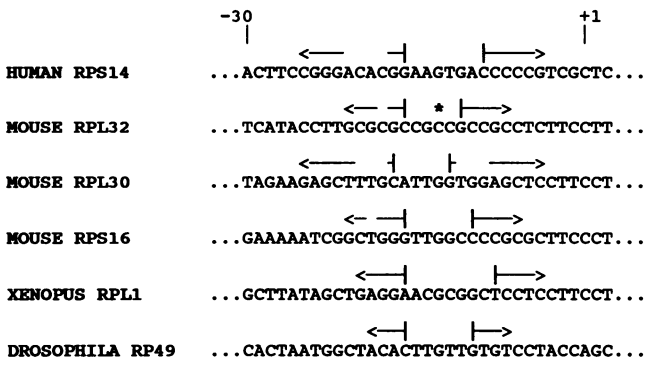

FIG. 9. Proximal upstream sequences in animal r-protein genes contain similar inverted DNA repeats. DNA sequences immediately upstream of the transcriptional initiation site (positions +3 to −30) in human RPS14 (37), mouse RPL32 (9), RPL30 (46), RPS16 (42), *X. laevis* RPL1 (22), and *D. melanogaster* RP49 (34) are aligned. Arrows are positioned above short sequences that form inverted repeat units. The asterisk above position −13 in murine RPL32 marks the 3′ breakpoint of a deletion that precludes normal RPL32 mRNA initiation (10).

Because the vast majority of S14 protein is associated with extremely long-lived ribosomal particles and the intracellular level of free S14 is undetectably low (2, 23), pulse-chase kinetic experiments to study S14 protein regulation in tissue culture cells are not yet technically feasible.

ACKNOWLEDGMENTS.

We thank Barbara Van Slyke and Marilynn Correll for their dedicated technical assistance. We acknowledge Susan Brown's and I-Tsuen Chen's gift of Chinese hamster RPL36a and RPS17 cDNAs. Dr. Brown's thoughtful editorial criticism of this manuscript also was greatly appreciated.

This research was supported by Public Health Service grant GM-23013 from the National Institutes of Health.

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