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Isolation and characterisation of a murine cDNA clone highly homologous to the yeast L29 ribosomal protein gene

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ABSTRACT

We report here the isolation of a murine cDNA clone (cCL3) which is homologous to the mRNA of the yeast ribosomal protein L29. Comparison of the deduced amino-acid composition of cCL3 to those known for rat ribosomal proteins indicates that this cDNA codes for mammalian ribosomal protein L27'. The gene corresponding to the cDNA is present at approximately 15 copies per genome, some of these probably representing processed pseudogenes. The cDNA hybridizes to an mRNA of 600 nucleotides from various mammals at high stringency, and to an avian transcript of the same size at low stringency. It has been suggested that L29 is involved in peptidyl transferase activity. The strong homology of mammalian L27' to yeast L29 suggests a function which has been conserved throughout evolution, and thus L27' may also be involved in peptidyl transferase activity.

INTRODUCTION

The eukaryotic ribosome is a highly complex organelle composed of four RNA species and 70-80 different ribosomal proteins (rp's, 1). The elucidation of the structure and function of the ribosome requires information on the structure of its various components. A great deal of information has been accumulated on the structure of the rRNAs, but much less is known about the ribosomal proteins. Although the amino-acid compositions of probably all of the rat rp's are known (2-4), primary structure information has been determined in only a few cases (5-10).

The complexity of ribosome structure also suggests the existence of a high degree of coordination in the expression of the ribosomal protein genes. Investigation of such controls, in particular at the transcriptional level, has been complicated by the fact that, in mammals, each ribosomal protein seems to be coded for by multiple genes, 7 to 20 in number (11). However, many of the gene copies appear to be inactive "processed" pseudogenes (5,6,10,12). In the cases of rp L30, L32 and S16, striking homologies have been found at the 5'-end of their genes, although they do not appear to be evolutionarily related. It was then postulated that these sequences could be involved in the coordinate

expression of these genes (10). Coordinate and differential expression of ribosomal proteins has been reported in regenerating rat liver (13) and during development of both Xenopus (14) and Drosophila (15) embryos.

cDNAs have been isolated for a few of the mammalian rp's and some non-mammalian rp cDNA clones are homologous enough to be used as probes (16). However, probes for the majority of the mammalian rp's are not yet available. In this paper we describe the isolation and characterisation of a murine cDNA clone which shows strong sequence homology to the yeast ribosomal protein L29. We have concluded that this cDNA clone corresponds to the murine L27' rp gene, based on amino-acid composition.

MATERIALS AND METHODS

Preparation and Analysis of DNA and RNA

Genomic DNA from F9 cells was prepared as described by Gross-Ballard et al. (17). After digestion with restriction enzymes, DNA was electrophoresed on 0.8% agarose gels and blotted to Pall Biodyne nylon membranes. After baking for 2 hours at 80°C, blots were prehybridized in 5 x SSC (1 x SSC:0.15 M NaCl, 0.015 M sodium citrate), 50% formamide, 1 x Denhardt's solution (18), 0.1% SDS and 200 µg/ml of denatured salmon sperm DNA at 42°C for 4 hours. Hybridization was in the same conditions, with the addition of 10% dextran sulphate and the nick-translated (19) ^{32}P-labelled probe for 16 hours. Washes were: 2 x 15' in 2 x SSC, 0.1% SDS at room temperature, 2 x 30' in 2 x SSC, 0.1% SDS at 65°C, and 2 x 15' in 0.1 x SSC at 65°C.

For Northern blot analysis, RNA was isolated according to Murphy et al. (20) with the exception of that from Lytechinus pictus (gastrula stage) which was kindly provided by Dr. Bruce Brandhorst. Poly(A) containing RNA was obtained by two passages through oligo-dT cellulose columns. The RNAs were fractionated in 1.2% agarose-2.2 M formaldehyde gels (19) with 10 mM phosphate (pH 6.5) as running buffer. After transfer to Pall membranes without any prior treatment of the gel, prehybridization, hybridization and washes were as described for Southern blotting. For low stringency hybridizations, both the prehybridization and hybridization were carried out at 37°C and the final wash was in 2 x SSC, 0.1% SDS at 55°C.

Sequencing of DNA

Sequencing of both strands of the cDNA insert was carried out by the method of Maxam and Gilbert (21). The extreme 5' region which was absent from the plasmid cCL3 (upstream of nt. 30, Fig. 1) was determined by primer extension as described by Henikoff et al. (22) with some modifications. The primer

used was a 21-mer complementary to nts. 121-141 of the cCL3 insert (Fig. 1). The oligonucleotide was labelled at the 5' end by using polynucleotide kinase and annealed to 20 µg of poly(A)$^+$ RNA overnight at 30°C in 0.4 M NaCl, 50 mM PIPES (pH 6.5), 1 mM EDTA and 80% formamide. The ratios of dNTP/ddNTP for A,T, G, and C were 1.9, 1.25, 5, and 5 respectively. AMV reverse transcriptase was from Life Sciences, Inc.

Hybridization selection and in vitro translation

Hybridization selection was done essentially as described by Parnes et al. (23) using 10 µg of cCL3 DNA and 50 µg of poly(A)$^+$ RNA from F9 cells. Selected RNA was translated in vitro in rabbit reticulocyte lysates.

RESULTS AND DISCUSSION

A cDNA library was prepared from polysomal poly(A)$^+$ RNA from F9 embryonal carcinoma cells (24) according to Gubler and Hoffman (25). After screening of the library with polynucleotide kinased RNA enriched for sequences specific to retinoic acid treated F9 cells, we isolated several clones of interest, one of which is described here.

The sequence of the insert of clone cCL3 was determined (Fig. 1) and was shown to contain an open reading frame of 444 nt with a 3' non-coding region of 38 nt. The sequence AUUAAA was found 14 nt upstream from the beginning of the poly(A) tail. This sequence is identical to the poly-adenylation signal of chick lysozyme mRNA (26). The cDNA insert starts at nucleotide 30 (position + 8). The sequence 5' to this position was determined by dideoxynucleotide interrupted primer extension using as primer an oligonucleotide complementary to positions 121-141. The 5' non-coding region was found to contain approximately 26 nucleotides, 22 of which were determined. The nucleotide -3 is a G, which is consistent with the generally accepted consensus of functional initiation codons (27).

Comparison of the obtained sequence to databanks of previously known sequences revealed a strong homology between cCL3 and the yeast ribosomal protein L29 (28). This homology is illustrated in Figure 1. Nucleotide homology was found to be 58% within the coding region, with both the 5' and 3' non-coding regions diverging strongly, as would be expected for homologous genes from such remote species. The protein coded for by clone cCL3 shows 62% strict homology to yeast L29. If conservative amino-acid changes are allowed, this homology increases to 74%. This homology is emphasized by a comparison of the plots of hydropathic index drawn according to Kyte and Doolittle (29) as shown in Figure 2. The plots indicate a high degree of similarity between the two

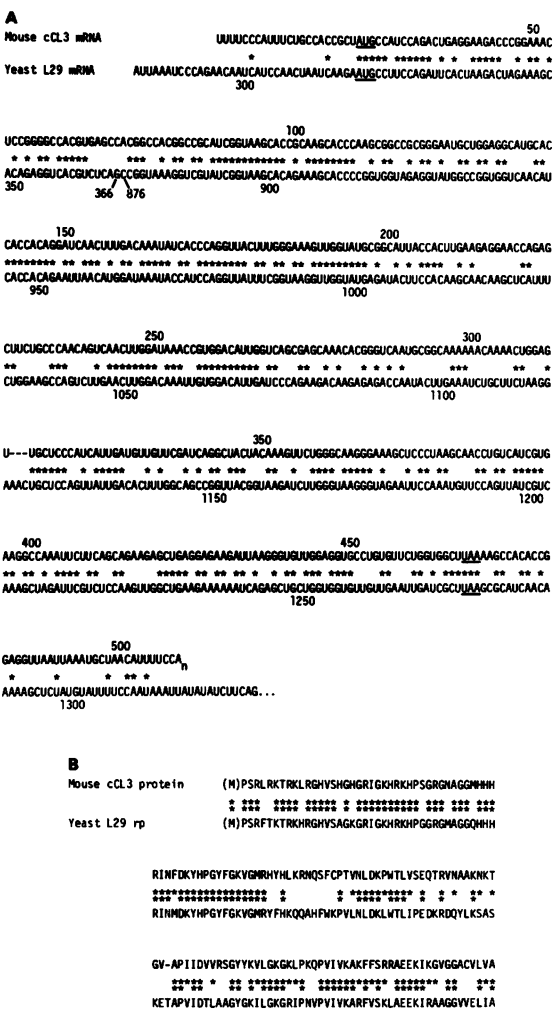

Fig. 1. Comparison of the nucleotide sequences of the murine cCL3 and yeast L29 rp mRNAs (A) and of the amino-acid sequences of the proteins that they encode (B). Initiation and termination codons are underlined. A gap of 3 nucleotides (corresponding to 1 amino-acid) was introduced at position 313 of the cCL3 sequence. In the protein sequences, double and single asterisks indicate identical and similar amino-acids respectively.

proteins. We thus concluded that clone cCL3 contains a cDNA copy of the mRNA coding for the murine equivalent of yeast rp L29. Homologies between mammalian and yeast ribosomal proteins have been reported before (7) but they were much weaker than observed here. This may indicate a strong evolutionary constraint

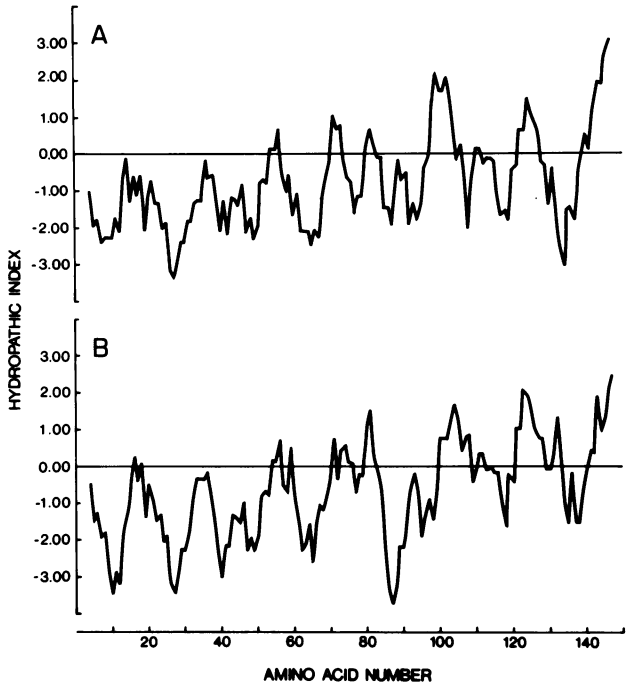

Fig. 2. Hydropathic index plots of cCL3 (A) and yeast L29 (B) proteins. Plots were drawn according to Kyte and Doolittle (29) with a window value of 6.

on this protein due to the importance of its function. Homology is particularly strong over the first third of the two sequences with 49 out the first 58 amino-acids (84%) identical between the two proteins. This region may thus be of particular functional importance.

The amino-acid contents of most of the rat ribosomal proteins have been determined (2-4). The accuracy of these data was confirmed by the determination of the primary structures of some of these proteins (5-10). The homology to the yeast rp L29 having already indicated that cCL3 probably coded for a ribosomal protein, we compared the amino-acid content of the protein coded for in its open reading frame to those known for rat rp's. The high contents of histidine, glycine and lysine of the cCL3 protein allowed us to rapidly eliminate all candidates with the exception of L27' (Table I). Considering all of the data, we believe that it is highly likely that cCL3 corresponds to the murine L27' mRNA.

The murine L27' would thus have a molecular weight of 16600 if unprocessed and of 16469 if the initiator methionine is removed as is the case in yeast

Table 1. Amino acid composition of cCL3 protein

Amino acid	cCL3 protein		Rat L27' rp
	Number of residues	% (moles percent) (a)	% (moles percent) (b)
Asp	3	2.1	8.0
Asn	6	4.2	
Glu	3	2.1	4.7
Gln	3	2.1	
Ala	8	5.6	5.7
Arg	14	9.7	9.7
Gly	18	12.5	13.7
His	11	7.6	7.2
Ile	6	4.2	3.6
Leu	8	5.6	6.3
Lys	18	12.5	12.9
Met	3	1.4 (a)	1.3
Phe	5	3.5	3.0
Pro	8	5.6	5.3
Ser	7	4.9	3.8
Thr	5	3.5	2.7
Tyr	5	3.5	3.4
Val	14	9.7	8.7
Cys	2	- (a)	-
Trp	1	- (a)	-
	148		

(a) To allow comparison, calculations were based on a total amino acid content of 144 residues: initial methionine, cysteine and tryptophan residues were omitted.
(b) From Tsurugi et al. (4)

(28). These values coincide well with the apparent molecular weight of the protein obtained by SDS PAGE analysis of the translation product of hybridization selected L27' mRNA which is about 16,500 (Fig. 3). This protein is highly basic containing 32 lysine and arginine residues as opposed to only 6 aspartate or glutamate. Codon usage is typical (30) with the exception of that of the histidine residues. The average frequencies of the occurrence of CAC and CAU as codons for histidine are of 0.58 and 0.42 respectively. In the L27' mRNA,

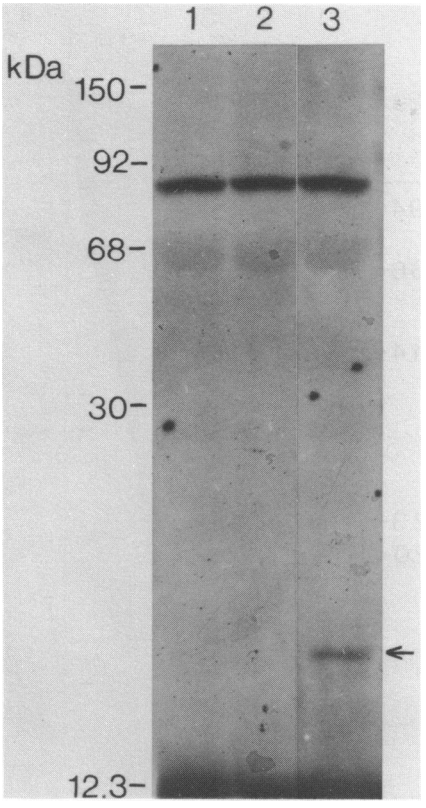

Fig. 3. In vitro translation of cCL3 mRNA. ^{35}S-labelled proteins were fractionated on a 12% SDS-polyacrylamide gel and visualized by autoradiography. Lanes contain the products of translation of: 1. no RNA. 2. RNA selected by pUC8. 3. RNA selected by cCL3. The arrow indicates the position of the 16.5 kDa cCL3 protein.

these numbers are 0.91 and 0.09, indicating a strong bias in favor of CAC.

All ribosomal protein genes for which probes are available to date have been shown to be present as multiple copies in the mammalian genome (11). L27' is no exception. Southern blot analysis of genomic DNA (Fig. 4) shows the presence of approximately 15 bands with any of 3 six-cutter restriction enzymes. This result was obtained whether DNA came from F9 cells (Fig. 4) or from mouse spleens (data not shown). The use of 5' and 3' subclones of the cDNA as probes also yielded a pattern identical to that obtained with the complete sequence (data not shown). L27' is thus represented by approximately 15 copies in the mouse genome. It is impossible at this stage to affirm what the organisation of these various copies of the L27' gene is. One result suggests that, as is

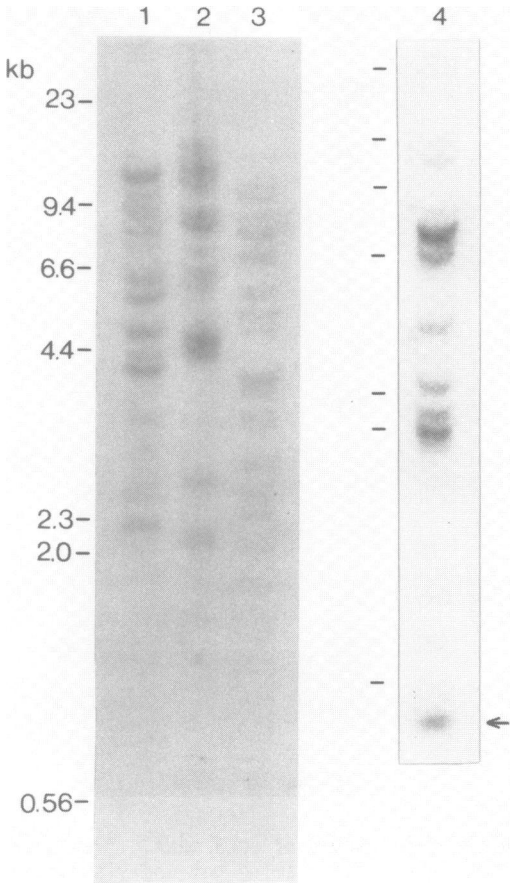

Fig. 4. Southern blot analysis of F9 genomic DNA. 10 μg of DNA per track were digested with EcoRI (lane 1), BamH1 (lane 2), PstI (lane 3) or MspI (lane 4, 20 μg of DNA). After transfer of DNA, blot was hybridized to ^{32}P-labelled, nick-translated cCL3 insert. The bars alonside lane 4 indicate the position of the λ HindIII markers. The arrow is at the position of the 430 bp fragment.

the case for other ribosomal protein genes, some of the L27' gene copies may be processed pseudogenes. In the L27' cDNA, there are two Msp I sites at positions 55 and 478 respectively. As these sites are near the two extremes of the mRNA sequence, they would probably flank to either side any introns that the gene may contain. If intronless copies were present, we would predict that the 432 bp band contained by Msp I digestion would be present in genomic Southern blots. In fact, if Msp I digested F9 DNA is hybridized to cCL3, such a band is

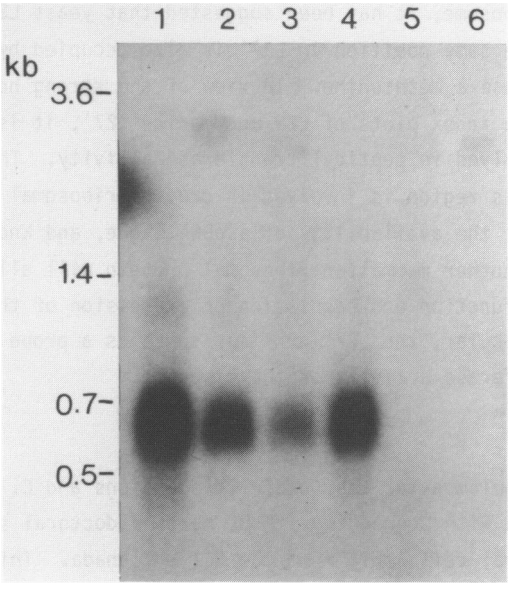

Fig. 5. Northern blot analysis of mRNA from different species. 2 µg of poly(A)$^+$ RNA were loaded per track except for tracks 5 and 6 where 4 µg were used. Lane 1: F9 cells (mouse); lane 2: 620.7 cells (hamster); lane 3: T98G cells (human); lane 4: Vero cells (monkey); lane 5: H32 cells (chicken); lane 6: gastrula stage Lytechinus pictus (sea urchin). After transfer of RNA, blot was probed with ^{32}P-labelled nick-translated cCL3 insert. Molecular weight markers are EcoRI cut pAT153 and TaqI cut pUC8.

detected (Fig. 4). This would indicate that some of the L27' gene copies are in fact processed pseudogenes.

Northern blot analysis revealed that cCL3 hybridized to an abundant mRNA of 600 nt in length in preparations of poly(A)$^+$ RNA from F9 (Figure 5) or other murine cells (data not shown). When cCL3 was hybridized to poly(A)$^+$ RNA from other mammals, a similar 600 nt band was observed under both stringent and non-stringent conditions. No signal could be found in tracks containing RNA from sea-urchins or chicken cells under stringent conditions, but a faint signal was observed with the chicken RNA under conditions of low stringency (data not shown).

We noted above that the first third of the sequence of L27' is particularly highly conserved relative to that of the yeast L29. In yeast, mutations in amino-acid 37 of the L29 rp, with replacement of the uncharged glutamine residue by a charged lysine or glutamate lead to cycloheximide resistance (28, 31). As the action of cycloheximide is to block the peptidyl transferase

activity of the ribosome, it has been suggested that yeast L29 is involved in this function. The same position in L27' is also occupied by an uncharged residue, in this case a methionine. In view of the strong homology and highly similar hydropathic index plots of L29 and murine L27', it is probable that the latter is also involved in peptidyl transferase activity. This is a further indication that this region is involved in crucial ribosomal functions.

In conclusion, the availability of a cDNA clone, and knowledge of the primary sequence of another mammalian ribosomal protein will allow further studies on the structure, function and regulation of expression of these essential proteins. In particular, the L27' cDNA may serve as a probe for the study of the peptidyl transferase activity of the ribosome.

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