Structure of *Xenopus laevis* ribosomal protein L32 and its expression during development

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ABSTRACT

cDNA clones for *Xenopus laevis* ribosomal protein L32 have been isolated and sequenced. The deduced amino acid sequence indicates that L32 is a basic protein of 110 amino acids, has a molecular weight of 12,603 and is homologous to the rat ribosomal protein L35. Using the cDNA clone as a probe to follow the expression of this gene during *Xenopus* development, it has been shown that the pattern of accumulation of this mRNA follows the one previously described for other ribosomal protein mRNAs during oogenesis and embryogenesis. The analysis of the utilization of L32 mRNA during embryogenesis shows that this is controlled by the translational regulation typical of other ribosomal protein mRNAs.

INTRODUCTION

The structure and expression of the genes encoding ribosomal proteins (r-proteins) in *Xenopus laevis* has been the subject of extensive studies in the past decade (for reviews see ref. 1 and 2). Partial cDNA corresponding to few r-protein mRNAs have been isolated and used as probes i) to isolate the corresponding genomic clones and analyse the gene structure, and ii) to study ribosome biosynthesis in developing *Xenopus* oocytes and embryos, and after microinjection of cloned r-protein genes. These studies have revealed that the synthesis of r-proteins is controlled by at least two types of regulation: a translational regulation which controls the efficiency of utilization of r-protein mRNA in response to the cellular needs for new ribosomes, and a post-transcriptional regulation operated by feedback of the r-proteins themselves, which controls processing and stability of r-protein transcripts.

These conclusions, however, are based on the analysis of only few r-protein genes. In fact, while partial cDNAs have been isolated for 10 of the about 80 *Xenopus laevis* r-proteins (3, 4), full length cDNAs have been sequenced for only five of them, L1, L5, L14, S8 and S19 (5–9) and the complete structure at the gene level has been determined only for L1 and L14 (5, 10). The pattern of expression during oogenesis and embryogenesis has been studied for r-protein L1, L14, S1, S8 (11, 12) and for L13, L15, L23 and S22 (4), but the gene dosage alteration by microinjections has been carried out only for L1 and L14 (13, 14). At this point it is important to extend our studies to the genes for other r-proteins, which may not all be controlled in an identical fashion.

With this purpose in mind we have started the study of r-protein L32. In this paper we present the complete nucleotide sequence of a full length L32 cDNA clone and the deduced aminoacid sequence. We present also the analysis of the L32 gene expression during *Xenopus* development, showing that the synthesis of this r-protein is translationally regulated.

MATERIALS AND METHODS

Biological materials

Xenopus laevis adults were purchased from Nasco (Wisconsin, USA). Oocytes were separated manually into six stages according to Dumont (15) and staging of embryos was according to Nieuwkoop and Faber (16).

Cell fractionation and RNA analysis

For polysome analysis, cytoplasmic extracts were prepared from 40–60 embryos of stages 15 and 32, centrifuged on 15–50% sucrose gradients and fraction precipitated as previously described (11). Total RNA extraction from oocytes or embryos, or from precipitated polysomal fractions, was performed essentially as described (17) and analyzed by Northern hybridization. RNA transfer on GeneScreenPlus membranes (Du Pont), prehybridization and hybridization were performed according to the GeneScreenPlus protocol.

Screening of the cDNA bank

A full length cDNA bank, constructed with mRNA from *Xenopus* embryos of stage 40 (18), was screened essentially as described (19) using as a probe the insert of plasmid pXom78 (3), corresponding to the 3′ portion of L32 cDNA.

DNA sequencing and computer analysis

Sequencing was performed mainly by the Sanger dideoxy method (20), and in part by Maxam and Gilbert method (21). Sequences were analyzed by using the IBI/Pustell package of software.

Quantification of autoradiography

X-ray films of Northen blots were quantified by analysis with an LKB Ultrascan XL laser densitometer.

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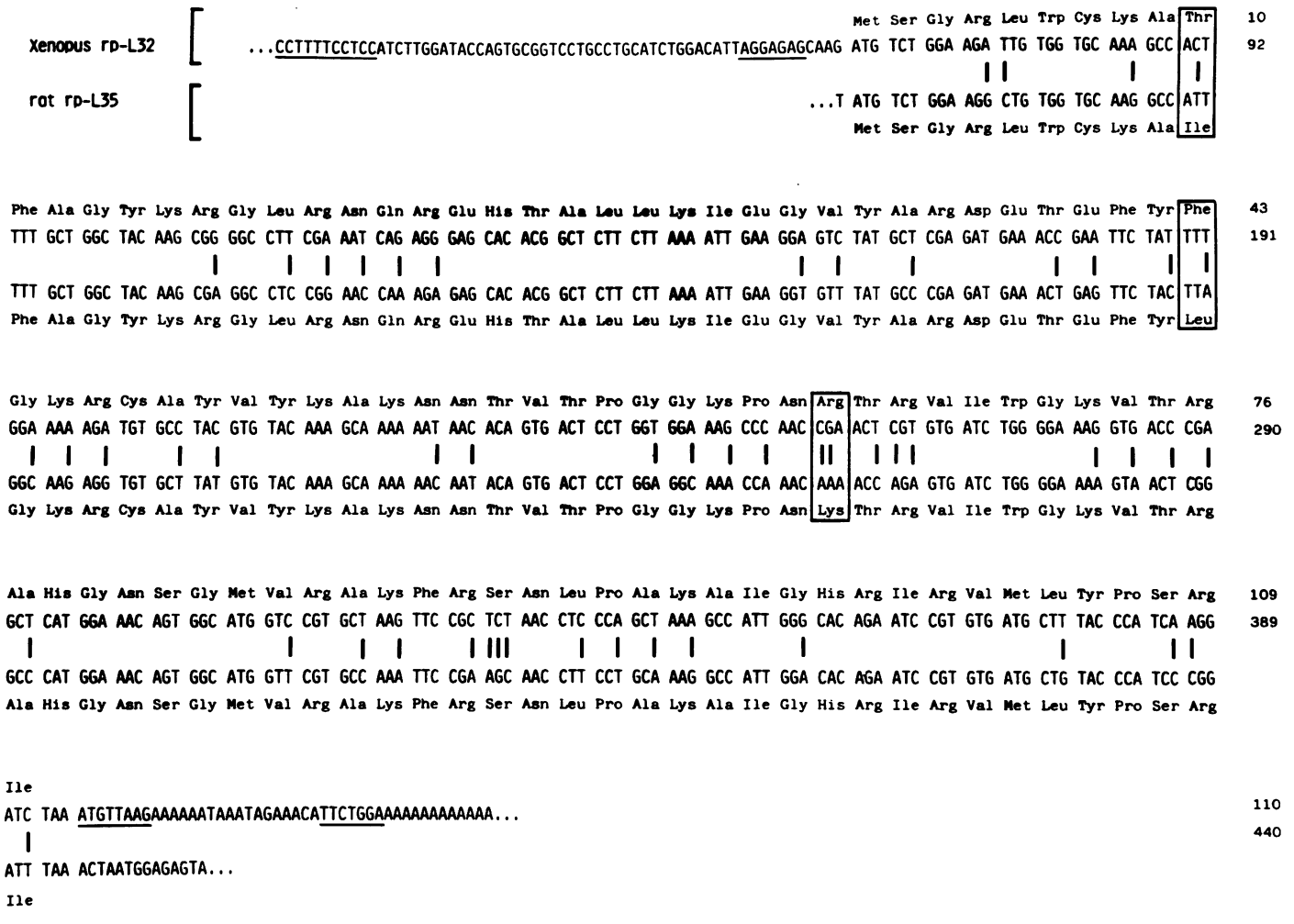

Fig. 1. Nucleotide sequence of *Xenopus* r-protein L32 cDNA and its derived amino acid sequence. For comparison the nucleotide and amino acid sequences of the homologous rat r-protein L35 are shown (22). Bars and boxes indicate nucleotide and amino acid substitutions respectively. The numbers on the right refer to the positions of the last nucleotide and the last amino acid in each line. Sequence motifs common to other *Xenopus* r-protein mRNAs are underlined in the 5′ and 3′ UTRs.

RESULTS

Structure of the *Xenopus* r-protein L32 cDNA

A full length *Xenopus laevis* cDNA bank was screened using as a probe a previously isolated cDNA fragment (3), corresponding to the C00H-terminal portion of the r-protein L32 (9). Eight independent positive clones were isolated and sequenced at their 5′ ends. The six longest cDNAs are probably complete at the 5′ end since all of them start at the same position with a run of pyrimidines typical of the *Xenopus* r-protein mRNAs up to now analyzed (see below). These six full length cDNAs were entirely sequenced and resulted to be derived from independent cloning of the same mRNA. Figure 1 shows the 440 nt long sequence of L32 cDNA. Besides the pyrimidine run at the 5′ end, this cDNA presents also other characteristic motifs in the 5′ and in the 3′ non coding regions, which have been observed in the other *Xenopus* r-protein mRNAs (8).

The deduced aminoacid sequence of *Xenopus* r-protein L32 consists of 110 residues, it has a Mr of 12,603 and shows, as expected, a strong basic character: there are 27 basic aa (Lys, Arg and His) and 5 acidic aa (Glu and Asp). Computer search on sequence data banks has revealed that this *Xenopus* r-protein L32 is homologous to the rat r-protein L35 (22), which is shown in Figure 1 for comparison.

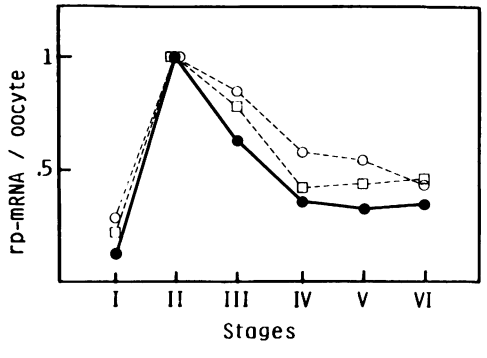

Fig. 2. Accumulation pattern of r-protein L32 mRNA during oogenesis (●). Total RNA corresponding to 2 oocytes of each stage has been analyzed by Northern blot hybridization and quantified by densitometric scanning of the autoradiograms; the average values obtained in two independent experiments are expressed relative to the highest one. For comparison the accumulation patterns of r-protein S8 mRNA (□) and L14 mRNA (○) are shown.

r-protein L32 mRNA accumulation during *Xenopus* oogenesis and embryogenesis

The above described cDNA has been used as a probe to follow the accumulation of r-protein L32 mRNA during *Xenopus*

oogenesis and embryogenesis. For this purpose total RNA corresponding to a fixed number of oocytes or embryos of different stages was analyzed by Northen blot hybridization. As a control (not shown) we determined the amount of total RNA in each sample which reproduced the well-known pattern of accumulation of ribosomes during oogenesis and embryogenesis.

The autoradiograms obtained in several Northern experiments on different batches of oocytes and embryos were quantified by densitometric scanning and the average values are plotted in Figures 2 and 3 respectively. For comparison the same filters were rehybridized with probes specific for r-proteins S8 and L14, whose accumulation patterns have been previously described (11).

Figure 2 shows that r-protein L32 mRNA is accumulated very rapidly at the beginning of oogenesis, reaches the highest value at stage II, and decreases afterwards. This accumulation pattern is similar to those of r-protein S8 and L14 mRNAs.

Figure 3 shows that at the beginning of embryogenesis the small amount of maternal L32 mRNA disappears in a few hours; after the mid-blastula transition (stage 8), the amount of r-protein L32 mRNA starts to increase again following the same pattern of accumulation of r-protein S8 and L14 transcripts.

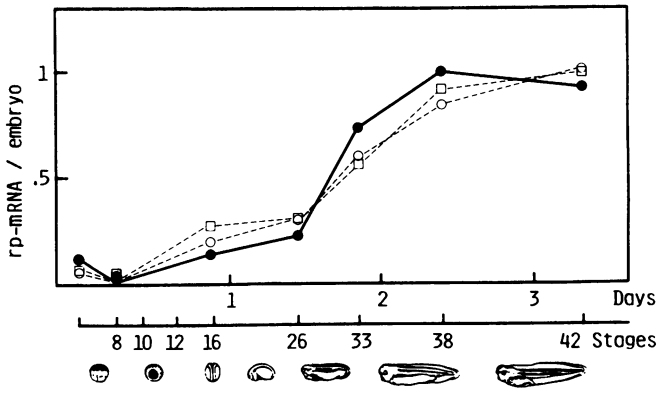

Fig. 3. Accumulation pattern of r-protein L32 mRNA during embryogenesis (●). Total RNA corresponding to 2 embryos of each stage has been analyzed by Northern blot hybridization and quantified by densitometric scanning of the autoradiograms; the average values obtained in two independent experiments are expressed relative to the highest one. For comparison the accumulation patterns of r-protein S8 mRNA (□ and L14 mRNA (○) are shown.

Polysome/mRNP distribution of r-protein L32 mRNA in *Xenopus* embryos

In order to determine the proportion of r-protein L32 mRNA translated during *Xenopus* embryogenesis, we have analyzed its distribution between polysomes and mRNPs in embryos of stages 15 and 32. Figure 4 shows the result of a typical experiment. The same filter was rehybridized with probes specific for r-proteins S8 and L14 for comparison, and with a probe for histone mRNA as an unrelated control. The autoradiograms of different experiments were quantified by densitometric scanning and the percentage of mRNAs on polysomes were calculated. It appears that at stage 15 about 20−30% of r-protein L32 mRNA is found onto polysomes, while at stage 32 the percentage increases to about 50−60%. This loading pattern is very similar to the one of r-protein S8 and L14 mRNAs, as already described (12). Histone mRNA is completely loaded onto polysomes at both stages.

DISCUSSION

Our understanding of the structure and expression of the genes coding for the about 80 r-proteins in *Xenopus laevis* is based on the analysis of very few of them. With the aim of expanding our knowledge to other proteins of this group we have undertaken the study of r-protein L32, for which only a partial cDNA had been isolated (3) and no expression analysis carried out. Thus we have isolated full lenght cDNAs in order to gain complete information about the structure of its corresponding mRNA and its deduced amino acid sequence. We have analysed a rather large number of clones in search of a cDNA corresponding to a second copy of L32 gene. In fact the r-protein genes up to now studied, like most other genes in *Xenopus laevis*, are present in two copies per haploid genome, due to a whole genome duplication occurred

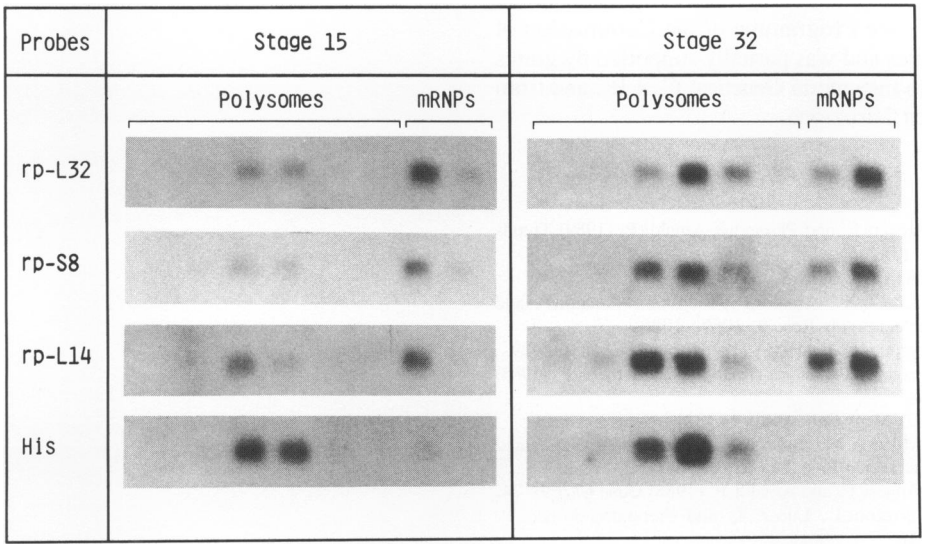

Fig. 4. Polysome/mRNP distribution of mRNA for r-protein L32 in *Xenopus* embryos. Cytoplasmic extracts from embryos at stage 15 and 32 have been fractionated on sucrose gradients. Eight fractions have been collected from each gradient, the RNA extracted and analyzed by Northern blot hybridization. For comparison the distribution of mRNA for r-proteins S8, L14 and for Histones (only the major band) is also shown.

in an ancestor of this animal species about 30 million years ago (23). The eight L32 cDNAs sequenced in the present study correspond to the same gene copy; this however is not sufficient to rule out the existence of a second copy of L32 gene, possibly with a low expression level. The cDNA sequence shows that the 5′ and 3′ untranslated regions (UTRs) of the r-protein L32 mRNA closely resembles the other *Xenopus* r-protein mRNAs up to now characterized (8). In particular, the 5′ UTR is rather short (62 nt), it starts with a typical run of pyrimidines, and contains a purines rich sequence (AGGAGAG); also the 3′ UTR is very short (32 nt) and it has the sequences ATGTTAAG and TTCTGGA already observed in mRNAs for other *Xenopus* r-proteins.

The comparison between the *Xenopus* r-protein L32 mRNA and the homologous rat r-protein L35 mRNA (22) shows that 54 nucleotide changes, out of 330 coding nucleotides, occurred since the two species diverged; 50 of them fall on silent sites, while the remaining ones cause three amino acid substitutions, indicating that the primary structure of this r-protein is under a strong selective pressure.

The level of r-protein L32 mRNA has been followed during *Xenopus* oogenesis and embryogenesis, and it turns out that the accumulation pattern of this transcript follows the general developmental expression of the other r-protein genes already analysed (4, 11). By analysing its distribution between polysomes and mRNPs at stages 15 and 32 of embryogenesis, we have also shown that this mRNA is translationally controlled in the same fashion as the mRNA for the other r-proteins (4, 12). This suggests that the typical structural features of the 5′UTR, shared by the L32 mRNA and the other r-protein mRNAs anlyzed, are involved in the translational regulation as demonstrated in the case of the mRNA for r-protein S19 (24).

The availability of a full length cDNA for the r-protein L32 can be now used to screen a genomic library in order to isolate the corresponding gene.

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REFERENCES

1. Amaldi,F., Bozzoni,I., Beccari,E. and Pierandrei-Amaldi,P. (1989) Trends Biochem. Sci. **14**, 175–178.
2. Wormington,W.M. (1988) Dev. Biol. **5**, 227–240.
3. Bozzoni,I., Beccari,E., Luo,Z.X., Amaldi,F., Pierandrei-Amaldi,P. and Campioni,N. (1981) Nucleic Acids Res. **9**, 1069–1086.
4. Baum,E.Z. and Wormington,W.M. (1985) Dev. Biol. **111**, 488–4989.
5. Loreni,F., Ruberti,I., Bozzoni,I., Pierandrei-Amaldi,P. and Amaldi,F. (1985) EMBO J. **4**, 3483–3488.
6. Wormington,W.M. (1989) Mol. Cell. Biol. **9**, 5281–5288.
7. Beccari,E., Mazzetti,P., Mileo,A.M., Bozzoni,I., Pierandrei-Amaldi,P. and Amaldi,F. (1986) Nucleic Acids Res. **14**, 7633–7646.
8. Mariottini,P., Bagni,C., Annesi,F. and Amaldi,F. (1988) Gene **67**, 69–74.
9. Amaldi,F., Beccari,E., Bozzoni,I., Luo,Z.X. and Pierandrei-Amaldi,P. (1982) Gene **17**, 311–316.
10. Beccari,E. and Mazzetti,P. (1987) Nucleic Acids Res. **15**, 1870–1872.
11. Pierandrei-Amaldi,P., Campioni,N., Beccari,E., Bozzoni,I. and Amaldi,F. (1982) Cell **30**, 163–171.
12. Pierandrei-Amaldi,P., Beccari,E., Bozzoni,I. and Amaldi,F. (1985) Cell **42**, 317–323.
13. Bozzoni,I., Fragapane,P., Annesi,F., Pierandrei-Amaldi,P., Amaldi,F. and Beccari,E. (1984) J. Mol. Biol. **180**, 987–1005.
14. Pierandrei-Amaldi,P., Bozzoni,I. and Cardinali,B. (1988) Genes Dev. **2**, 23–31.
15. Dumont,J.N. (1972) J. Morphol. **136**, 153–180.
16. Nieuwkoop,P.D. and Faber,J. (1973) Normal Table of *Xenopus laevis* (Daudin). North-Holland, Amsterdam.
17. Probst,E., Kressmann,A. and Birnstiel,M.L. (1979) J. Mol. Biol. **135**, 709–732.
18. Stutz,F. and Spohr,G. (1986) J. Mol. Biol. **187**, 349–361.
19. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Press, Cold Spring Harbor.
20. Sanger,F., Miklen,S. and Coulson,A.R. (1977) Proc. Natl. Sci. U.S.A. **74**, 5463–5467.
21. Maxam,A.M. and Gilbert,W. (1980) Methods Enzymol. **65**, 499–560.
22. Tanaka,T., Wakasugi,K., Kuwano,Y., Ishikawa,K. and Ogata,O. (1986) Eur. J. Biochem. **154**, 523–527.
23. Bisbee,C.A., Baker,M.A., Wilson,A.C., Hadji-Azimi, I. and Fishberg,M. (1977) Science **195**, 785–787.
24. Mariottini,P. and Amaldi,F. (1990) Mol. Cell. Biol. **10**, 816–822.