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Expression of the gene for ribosomal protein L1 in *Xenopus* embryos: alteration of gene dosage by microinjection

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Cloned gene for *Xenopus* ribosomal protein L1 was injected into fertilized eggs, and its expression was analyzed during the period of embryo development when the mRNAs produced by the endogenous ribosomal protein genes are still silent due to a translational control. The injected genes replicated extensively, and a 10-fold excess of L1 mature transcript accumulated in the embryo. This was accompanied by a small amount of incompletely processed L1 RNA that still contained one out of nine introns, a molecule never observed in normal conditions. The excess mature L1 mRNA was distributed between polysomes and messenger ribonucleoproteins (mRNPs) in the same relative proportion observed in control embryos of the same stage. Therefore, more L1 mRNA was loaded onto polysomes and caused the appearance of L1 protein when this was not yet detectable in control embryos. The results suggest a relationship between the excess amount of L1 protein and the alteration in processing of its transcripts.

[Key Words: *Xenopus laevis*; ribosomal proteins; regulation; gene injection]

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In previous studies we analyzed the expression of ribosomal protein (r-protein) genes at various regulatory levels during the development of normal *Xenopus* embryos and of anucleolate mutants homozygous for a deletion of the rRNA gene cluster. The general view outlined by our studies implies that at least two types of control mechanisms are involved in the regulation of r-protein synthesis in the *Xenopus* system. Control at the level of translation establishes what fraction of r-protein mRNA (rp-mRNA) must be loaded onto polysomes, and this control seems to respond to the need for new ribosomes, as occurs in early embryogenesis when the rp-mRNA is kept silent on messenger ribonucleoproteins (mRNPs) before being translated. Control at the post-transcriptional level regulates the stability of the r-protein transcripts and seems to be related to an overproduction of r-proteins relative to the amount needed for ribosome assembly (Pierandrei-Amaldi et al. 1982, 1985a).

At present we have no positive clues about the mechanisms responsible for this kind of translational control, which is also reported in other eukaryotic systems (Geyer et al. 1982; Kay and Jacobs-Lorena 1985; Schimdt et al. 1985); although we have ruled out the possibility that it is of an autogenous nature (Pierandrei-Amaldi et

al. 1985a,b). This is at variance with prokaryotes, in which r-proteins, if synthesized in excess, specifically block further translation of their own mRNA (Nomura et al. 1984). On the other hand, at least for r-protein L1, we have some indications on the mechanism that presides over the post-transcriptional control. These come from experiments in which the gene dosage for r-protein L1 has been altered by injection of an excess of the corresponding cloned gene into the germinal vesicles of *Xenopus* oocytes (Bozzoni et al. 1984). The chromosomal genes are already engaged in an active synthesis of the r-proteins necessary for the ribosomal accumulation typical of oogenesis; upon injection, they actively transcribe the excess L1 genes, but do not produce the corresponding mature mRNA while accumulating a large amount of a specific precursor RNA which still retains two out of nine intron sequences (Bozzoni et al. 1984). We have also found that this specific block of processing is partially prevented by coinjecting, together with L1 genes, antibodies raised against L1 protein (Pierandrei-Amaldi et al. 1987). These results suggest the possibility that an autogenous regulation, operated directly or indirectly by the final product (that is, L1 protein), controls the amount of mature mRNA by blocking a specific step of its transcript processing. Thus the oocyte, which is

synthesizing r-proteins in the proper amount for its ribosome accumulation, would prevent an excess production of the L1 protein component of the ribosome.

If this interpretation is correct, one would expect different behavior when extra copies of cloned L1 genes are injected into fertilized eggs and their expression is analyzed during the first part of embryogenesis. In fact in this period, schematically illustrated in Figure 1, rp-mRNA is synthesized and accumulated by the normal embryo after the midblastula transition, but it is scarcely translated up to stage 26; r-proteins start to be actively synthesized only at later stages, when 70–80% of rp-mRNA becomes recruited on polysomes (Pierandrei-Amaldi et al. 1982; Baum and Wormington 1985). Therefore, at variance with the oocyte, early embryo cells are devoid of free r-proteins. In this case one can predict that the excess transcripts produced upon L1 gene injection should be processed normally.

With this in mind we have injected cloned L1 genes into fertilized eggs and analyzed their expression during the "pretranslational period of rp-mRNA." To prevent impairing of the normal regulatory processes involved in the production of r-protein L1, we preferred not to modify the gene to be injected. In this way the transcripts cannot be distinguished from the endogenous ones, but this is not relevant in this study where an overproduction of normal L1 transcripts was desirable.

Results

Fate of injected DNA

The isolation and characterization of a cloned *Xenopus* genomic fragment containing the gene for r-protein L1 has been described (Bozzoni et al. 1982; Loreni et al. 1985). pL1, a subclone in pBR322 carrying an insert of 12 kb (Bozzoni et al. 1984), was used for microinjection (Fig. 1). Supercoiled pL1 DNA was injected into *Xenopus* fertilized eggs in amounts ranging from 50 to 400 pg per egg, corresponding to the order of a few million gene copies. Some eggs were injected only with buffer and used as controls. The amount of DNA injected was crucial for the viability of the embryos. In fact 300 pg or more was lethal and embryos did not pass gastrulation; with lower amounts of DNA, viability progressively increased. It was found that embryos injected with 50 or 100 pg are highly viable but often replicate the injected DNA poorly, whereas a combination of good replication efficiency and viability was obtained using 200 pg of pL1 DNA, as also observed by other authors (for instance see Rusconi and Schaffner 1981). Thus, 200 pg was the amount of DNA routinely used for injections. It must be pointed out that a certain degree of variability in the response to the injected DNA was observed among different batches of eggs. Figure 2 shows an example of the replication pattern of the injected L1 DNA at different stages of development. Total nucleic acid was extracted from buffer and DNA-injected embryos of various developmental stages; aliquots corresponding to one embryo were analyzed by Southern blotting and hybridized to L1 probe. To allow better quantitation, the extracted DNA

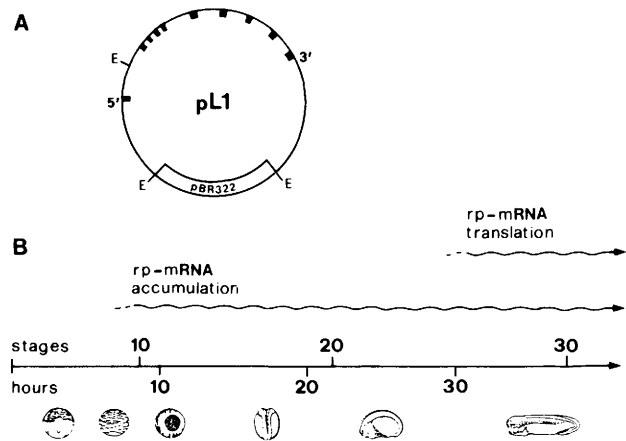


Figure 1. (A) Schematic representation of the recombinant plasmid pL1, used for microinjections. A 12-kb *Xenopus* genomic fragment containing the L1 gene is inserted in the *EcoRI* site of pBR322 (Bozzoni et al. 1982; Loreni et al. 1985). The 5' and 3' ends of the L1 gene are indicated; the 10 exons are represented by black boxes. (E) *EcoRI* restriction site. (B) Timing of r-protein gene expression during *Xenopus* development (Pierandrei-Amaldi et al. 1982). rp-mRNA starts to be accumulated around stage 10, but is underutilized until stage 26. The experiments described in this paper have been carried out between these two stages. Embryo stages are according to Nieuwkoop and Faber (1956).

was digested with *Bam*HI, which cleaves the injected DNA at two sites, one in the vector and the other within the gene (Fig. 2A). The endogenous chromosomal L1 gene also gives two bands, but of different mobilities, visible in Figure 2A in lanes d of the first and second sets of samples; in lane d of the third set of samples (200 pg) they are covered by the large amount of replicated DNA. In some experiments, such as the one shown in Figure 2, the amount of DNA increased up to stage 35; in other experiments the maximum was reached around gastrulation (stage 10), but persisted in the following stages. The extent of L1 DNA increase with respect to the DNA injected varied between 10- and 50-fold, as determined by densitometric analysis. However, for the purposes of this study the relevant point is the increment of the number of L1 gene copies with respect to the endogenous one. The difference between the amount of the endogenous L1 gene and the amplified DNA can be appreciated by comparing the control and the injected embryos at stage 35 (lanes d in Fig. 2). In fact the injected embryos contain several tens of excess copies, as compared with controls, which at this stage only have enough cells (about 3×10^5) to make a single copy gene just visible (Fig. 2A, first panel, lane d). Undigested DNA from the embryos injected with 200 pg was also analyzed to show the conformational state of the amplified copies (Fig. 2B). As previously described for other injected genes (Rusconi and Schaffner 1981; Andres et al. 1984; Bendig and Williams 1984; Etkin et al. 1984), the supercoiled molecules are relaxed immediately after injection. Later some of the amplified sequences appear as supercoiled monomers, and most of them appear as

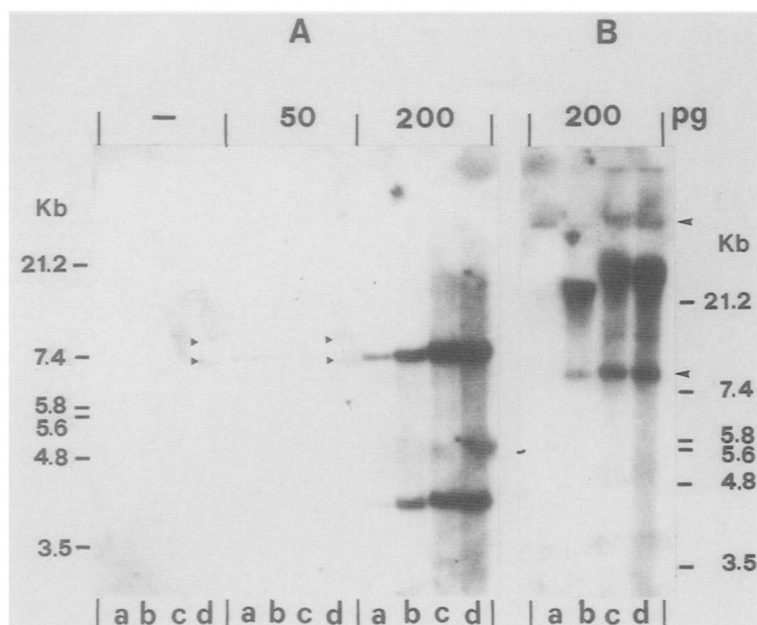


Figure 2. Replication in developing embryos of L1 genes injected into fertilized eggs. At various stages DNA was extracted, analyzed by Southern blotting, and hybridized to an L1 probe. Each lane was loaded with the DNA equivalent to one embryo, digested with *Bam*HI (A), or undigested (B). The amount of DNA injected is indicated at the top and the stages are analyzed at the bottom: before first cleavage (lanes a); stages 12, 31, and 35 (lanes b, c, and d respectively). In A arrowheads point to bands corresponding to the endogenous gene; in B arrowheads indicate the position of relaxed (upper) and supercoiled (lower) plasmid. Molecular weight markers (λ DNA digested with *Eco*RI) are indicated in kilobases.

high-molecular-weight forms that have the same mobility as the endogenous bulk genomic DNA (not shown). The complete conversion of these high-molecular-weight forms into the two bands expected after digestion with *Bam*HI (Fig. 2A) indicates that the plasmid is present as concatenates up to the tailbud stage. We have not investigated whether they remain episomal or integrated into the host DNA.

Transcription and accumulation of L1 RNA in injected embryo

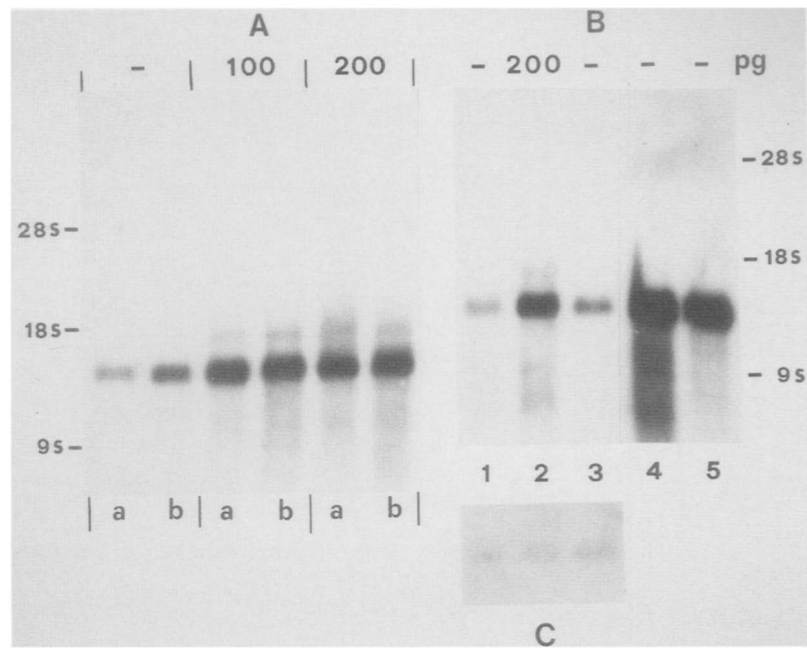
Once we had established that a large amount of template was produced after injection of the L1 gene, we checked if it was transcribed and how its transcripts were utilized. Poly(A)⁺ RNA from buffer and DNA-injected embryos was analyzed by Northern blotting and hybridized to L1 probe. We observed that up to stages 7–8, although there was a considerable increase of the injected DNA, no L1 transcripts were detectable, but around stage 10 an excess amount of L1 transcript started to accumulate in injected embryos as compared with controls (not shown). No hybridization was found in poly(A)⁻ RNA.

We have focused our attention in particular on the developmental period that precedes stage 26, when the rpmRNA is poorly translated. Figure 3A shows an example of L1 transcript accumulation at stages 14 and 20 in embryos injected with L1 DNA. The amount of transcript is 5- to 10-fold higher than in buffer-injected embryos as determined by densitometric analysis. A correlation between transcript accumulation and DNA increase was constantly observed. Most of the L1 transcript has the same mobility as mature L1 RNA of control, but a slower migrating band is observed in the RNA from injected embryos. This band has never been observed in RNA from controls of the same stage or of later stages even after overexposure of the autoradiographs, and it is

not merely related to the higher signal given by the RNA from injected embryos (Fig. 3B, lane 2 as compared with lane 1). In fact in lanes overloaded with RNA from normal embryos and oocytes, which gives a very high signal, the slower band is absolutely absent (lanes 4 and 5). The increase of transcript is specific for L1 sequences; in fact if the same filter was hybridized a second time to a probe for the r-protein L14, there was no difference between control and injected embryos (Fig. 3C).

The presence of heavier L1 transcripts after injection of the L1 gene was formerly observed in oocytes, and it was demonstrated that they still retained either the second and third introns or only the third (Bozzoni et al. 1984; Caffarelli et al. 1987). To establish if we were dealing with the same premature L1 RNA, we compared by Northern blotting and S1 analysis the RNA from injected oocytes and embryos. Figure 4 shows a Northern blot analysis of poly(A)⁺ RNA from embryos injected with the L1 gene (lane a) run in parallel with similarly treated oocytes (lane b), which are known to accumulate L1 transcripts still retaining the second and third introns. It appears that in the embryo the premature transcripts migrate somewhat faster than the corresponding one in the oocyte. To define the structure of such RNA species precisely, we compared the RNAs from L1-injected embryos and oocytes by S1 analysis (Fig. 5). The probe utilized was a 813-bp-long *Xba*I-*Pvu*II fragment that covers 633 bp of the L1 gene (from the *Xba*I site inside intron 3 to the *Pst*I site inside exon 2). The remaining 180 bp belong to the vector from which the probe utilized was a 813-bp-long *Xba*I-*Pvu*II fragment in the *Xba*I terminus to map the sequences upstream to this site, thus allowing the identification of transcripts that contain either intron 3 alone or introns 2 and 3. From the figure it appears that the RNA from injected oocytes gives two bands of S1 protection: a 633-bp fragment corresponding to protection from the *Xba*I site to

Figure 3. Accumulation of L1 transcripts in injected embryos. (A) Poly(A)⁺ RNA was prepared from groups of embryos of two stages; the equivalent of two embryos was separated on Northern gels, blotted, and hybridized to an L1 probe. The amount of DNA injected is indicated at the top, and the stages are indicated at the bottom: stage 14 (lanes a) and stage 20 (lanes b). (B) Poly(A)⁺ RNA from two embryos of stage 20 injected with buffer (lane 1) and pL1 (lane 2) is compared with RNA from the same number of buffer-injected embryos of stage 30 (lane 3), with RNA from 40 (20-fold) buffer-injected embryos of stage 20 (lane 4), and 0.5 μ g of poly(A)⁺ RNA from uninjected *Xenopus* oocytes (lane 5). (C) Lanes 1, 2, and 3 of the experiment shown in (B) were rehybridized to a probe for L14.



the *Pst*I site and a 323-bp fragment corresponding to protection from the *Xba*I site to the 5' border of exon 3. These fragments correspond to the protection of precursors containing intron 3 or introns 2 and 3. The RNA from L1-injected embryos contains mainly the precursor with intron 3; after a long exposure the precursor containing the two introns can also be visualized. This result demonstrates that the slower migrating band observed in Northern blots is indeed a precursor RNA containing at least intron 3. A similar analysis, performed using a probe 3'-end-labeled in the *Bst*EII site inside exon 2, covered the same region as the previous one and allowed the mapping from exon 2 downstream. This analysis showed that no precursors containing only intron 2 are present in oocytes or embryos (not shown). This finding indicates that intron 2 can still be spliced from the precursor RNA, though with lower efficiency with respect to the other introns, whereas the removal of intron 3 seems to be a major limiting step in the production of mature L1 RNA.

Translation of the excess L1 mRNA

As previously mentioned, in early embryogenesis the rp-mRNA is regulated by translational control (Pierandrei-Amaldi et al. 1982; Baum and Wormington 1985). Therefore it is of interest to know if the excess of mature L1 mRNA present in the injected embryos is used during this period. Embryos injected with buffer and DNA were labeled with [³⁵S]methionine around stage 18 to see if L1 protein was synthesized before its time. Labeling was carried out for 45 min, since it is known from previous experiments that unused r-proteins are unstable and are degraded with a half-life of about 1 hr (Pierandrei-Amaldi et al. 1985a). To reduce the effect of individual variability we analyzed groups of eight embryos, injected

with either buffer or DNA. At the end of incubation with [³⁵S]methionine, each group was homogenized in conditions suitable to preserve nucleic acids and proteins and quickly divided into three parts, which were processed for analysis of DNA, RNA, and proteins (see Materials and methods). Thus, it was possible to know for each group of embryos the relationship among template, transcript, and protein product.

Proteins were acid-extracted, loaded on gels together with purified *Xenopus* r-proteins as internal standard, and analyzed by two-dimensional gel electrophoresis and fluorography. Figure 6B shows the typical pattern of proteins synthesized at stage 18 of normal development, when histones and some unidentified spots are constantly found, but r-proteins are not yet detectable. In the gel loaded with protein synthesized by embryos of

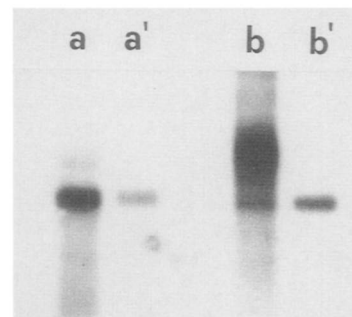


Figure 4. Comparison of transcripts produced by embryos and oocytes injected with the L1 gene. Poly(A)⁺ RNAs from stage 20 pL1-injected embryos (a), stage 20 buffer-injected embryos (a'), stage VI pL1-injected oocytes (b), and stage VI buffer-injected oocytes (b') were analyzed by Northern blot with an L1 probe. The gel was loaded with the RNA equivalent of two individuals.

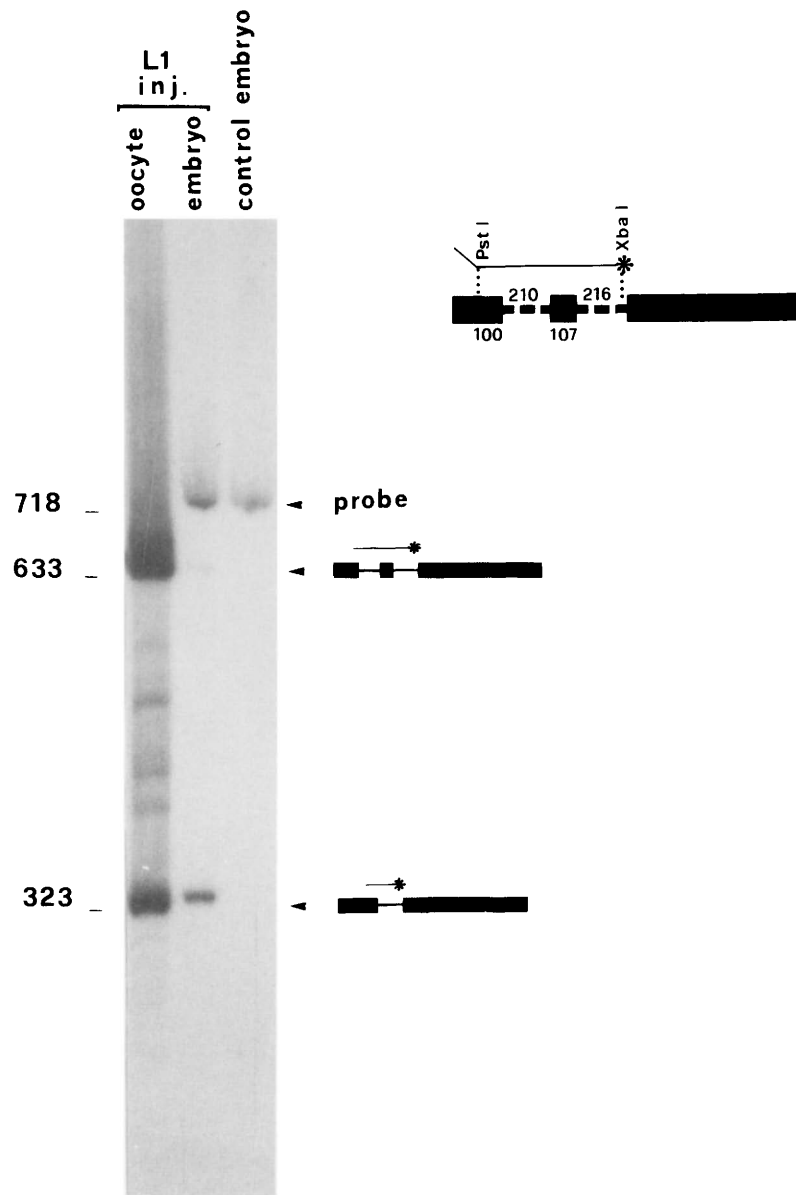


Figure 5. S1 analysis of RNA from embryos and oocytes injected with the L1 gene. The protected RNA from 10 embryos injected with buffer and pL1 and from 10 oocytes injected with pL1 is shown. (*Upper right*) The probe labeled at the 5' end (*) and the precursor region which it recognizes (still containing introns 2 and 3); (*below*) the structure of the two precursor forms revealed by the S1 protection.

the same stage injected with L1 DNA, the same pattern of control is observed except for the appearance of L1 protein (Fig. 6A). To show the position of r-proteins in this kind of gel, a later-stage pattern, when all r-proteins have started to be synthesized, is presented in Figure 6C (Pierandrei-Amaldi et al. 1982). Most of the faint spots present in this panel and absent in A and B are r-proteins as determined by comigration with stained internal standards in this gel optimized for basic proteins. We have checked that the RNA corresponding to the sample shown in Figure 6A contained a large amount of mature L1 transcript accompanied with the band corresponding to premature L1 RNA. A quantitation of the relative in-

tensity of the autoradiographic signals was carried out by densitometric analysis of the fluorographs of this and other similar experiments. The intensity of the L1 spot was normalized toward other spots considered as standards. The increase in protein L1 in injected embryos was calculated to be 5- to 10-fold over the controls that were overexposed to make the L1 spot barely visible.

Distribution of the excess L1 RNA between polysomes and mRNPs

Considering that the translational control of rp-mRNA observed in developing *Xenopus* embryos operates by

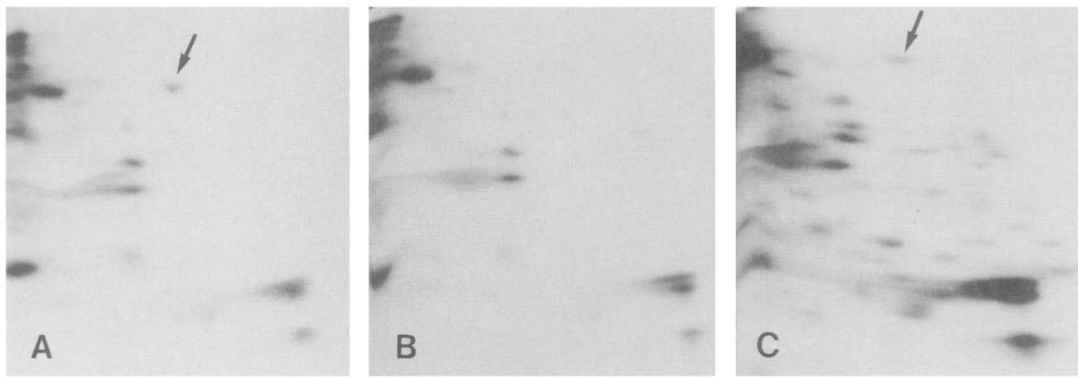


Figure 6. Fluorographs of acrylamide gels loaded with proteins synthesized by embryos injected with the L1 gene. Embryos injected with pL1 (A) and buffer (B) were labeled at stage 18 with [35 S]methionine; proteins were extracted and the equivalent of three embryos was run on two-dimensional acrylamide gels together with purified *Xenopus* r-proteins and fluorographed. For comparison a pattern of proteins synthesized by older embryos (stage 28), when r-proteins become visible, is shown (C). Arrows point to r-protein L1. Identification of labeled L1 and other r-proteins was determined by comigration with stained *Xenopus* r-proteins used as internal markers.

changing the distribution of this mRNA between polyosomes and mRNPs, it was of interest to know how the excess of L1 RNA was distributed between the two cellular compartments of injected embryos. Groups of buffer- and DNA-injected embryos were collected around stage 18 when, as mentioned above, in normal embryogenesis the great part of rp-mRNA is on mRNPs. Nuclei were removed and the cytoplasmic extracts were separated on sucrose gradients: each gradient was loaded with material corresponding to 10 embryos. Gradient fractions were collected in polyosomes and mRNP pools and the RNA extracted as described in Materials and methods. Poly(A)⁺ RNA was then analyzed by Northern blotting and hybridized to a probe for L1. (Purification of poly(A)⁺ RNA was necessary, since a certain amount of injected plasmid DNA could be present in the cytoplasm and disturb the analysis.) Figure 7 shows the Northern blot of poly(A)⁺ RNA from stage 18 polyosome and mRNP fractions of buffer- (A) and DNA-injected (B) embryos. It can be observed that L1 transcript is much higher in B than in A; however, a densitometric analysis of the films of this and other similar experiments, carried out at stages 18 and 23, has shown that the relative amount of L1 mRNA localized on polyosomes in injected embryos is approximately the same as in controls, namely around 10% at stage 18 and somewhat higher at stage 23. We have observed that the L1 RNA migrates as a single band in all the cytoplasmic fractions, whereas the premature L1 RNA was found only in the nuclear fraction (not shown). The same filter was rehybridized to a probe for protein L14; there was no difference in the amount or distribution of L14 mRNA in control and injected embryos.

Discussion

Taking advantage of the peculiar expression pattern of r-protein genes in the early phase of *Xenopus* development, when rp-mRNAs are accumulated but underutilized for translation, we have increased the gene dosage

for r-protein L1 to see how this alteration would interfere with the normal expression program of this gene. Injection of the cloned gene in fertilized eggs was a useful tool to achieve this.

We have shown here that the plasmid containing a *Xenopus* 12-kb genomic fragment with the L1 gene is efficiently replicated and can persist in the embryo up to hatching (stage 35), apparently as high-molecular-weight concatenates. Increase of L1 gene as compared with control embryos was, after gastrulation, between 10- and 50-fold as determined by densitometric analysis. Since the injected DNA replicated so much and persisted long enough for our purpose, we have not tried linearization of the plasmid which, for other DNAs, has been described to improve replication, persistence, and expression (Mohun et al. 1986; Wilson et al. 1986). Although in our case the DNA was not linearized, it is assumed that it is the high-molecular-weight form that produces ex-

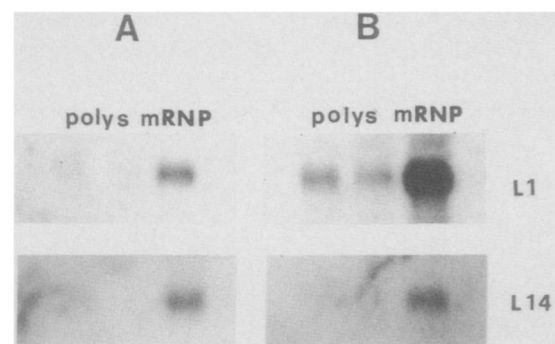


Figure 7. Distribution of L1 mRNA on polyosomes and mRNPs in buffer- and pL1-injected embryos. Cytoplasmic extracts from buffer- (A) and pL1-injected (B) embryos of stage 18 were fractionated on sucrose gradients. The regions corresponding to polyosomes and mRNPs were pooled and the corresponding RNA was analyzed by Northern blot hybridization with L1 probe. As a control, the same filter was rehybridized to an L14 probe.

pression, as indicated by these previous authors. Thus, different DNAs might show different behavior: in fact when we injected a circular plasmid carrying a gene for another r-protein we observed that it replicated less efficiently than L1, remained mostly supercoiled, and resulted in lower expression (P. Pierandrei-Amaldi et al. unpubl.). Moreover when a gene specifically expressed at gastrula was injected in supercoiled form, it was not significantly amplified and it was correctly transcribed in the embryo (Krieg and Melton 1985).

The large increase of L1 DNA is accompanied by a corresponding increase of the transcript. The excess L1 RNA starts accumulating around gastrulation, as in control embryos. In stages immediately following, namely in the period of time when in normal development the rp-mRNA is very poorly translated, the amount of L1 transcript was 5- to 10-fold higher than in controls. Most of the excess L1 transcript comigrates with mature L1 mRNA, and it is responsible for the early appearance of detectable L1 protein in the injected embryos. An interesting feature has been observed by analyzing the distribution of the excess L1 mRNA between polysomes and mRNPs in injected embryos and comparing it with the distribution of L1 mRNA in control embryos. It was found that this mRNA, although present in higher amount, has a relative distribution between the two compartments similar to controls, and typical of the developmental stage at least for the stages analyzed. This result provides an interesting clue about the mechanism involved in the translational regulation previously described for rp-mRNA in *Xenopus* development; namely, that the percent, rather than the amount, of rp-mRNA to be loaded onto polysomes is regulated. A similar result was obtained in *Drosophila* embryos injected with an r-protein gene (M. Jacobs-Lorena, pers. comm.).

In injected embryos the described increase of mature L1 mRNA is accompanied by the appearance of a small amount (about 5–10%) of larger L1 transcripts. These turned out to be premature forms of L1 RNA which still retained the third and sometimes also the second of the nine intron sequences, and are localized in the nucleus. These premature forms are evident exclusively in L1-DNA-injected embryos, which have more copies of this gene and were never observed in physiological conditions. It is unlikely that the appearance of the premature form is due to an overloading of the splicing system, as the excess L1 transcripts are very few compared with total ones (endogenous mRNA for each r-protein is about 0.1% of total poly(A)⁺ RNA). Moreover, except for introns 2 and 3, the other seven introns of L1 primary transcripts are processed normally. Furthermore, we have never observed any effect on the processing of the endogenous transcript for another r-protein (L14) in embryos injected with L1 genes.

Premature L1 RNA was first described in oocytes microinjected with the same L1 gene. Most L1 transcripts were not fully matured and retained the same introns; consequently no excess L1 protein was produced (Bozzoni et al. 1984). As an interpretation of that result it was postulated that in the oocyte, which is actively syn-

thesizing r-proteins, L1 protein would prevent the production of excess amounts of itself by specifically blocking the maturation of the corresponding RNA. This block was in fact partially prevented by injection of antibodies against L1 (Pierandrei-Amaldi et al. 1987). The results described here indicate that, on the contrary, most of the excess L1 transcript goes through maturation in early embryogenesis, causing the early appearance of L1 protein. After examining the results of several experiments, we propose that the excess L1 transcript is allowed to mature normally as long as the system is devoid of free L1 protein. When a certain amount of the excess L1 mRNA is loaded on polysomes, a corresponding amount of L1 protein is produced, too early to be assembled in the nucleus with the other ribosomal components which are not yet available. This unused L1 protein would make the system somewhat aware of its excess synthesis and would determine a block in the processing of the L1 transcripts that probably will take the way of degradation. A comparable situation was previously observed in the anucleolate embryo, which begins to decrease the level of its rp-mRNA only when the r-proteins start to be actively synthesized, namely around stage 30 (Pierandrei-Amaldi et al. 1985a). In this case only the final effect of degradation appeared, whereas in the injected embryo it was possible to make evident the intermediate steps (unprocessed transcripts) because of the overloading of the system.

In conclusion, the results described above support our previous interpretation that the expression of the L1 gene is regulated at a post-transcriptional level by an autogenous mechanism that operates at a specific step of transcript maturation, thus controlling the stability of its RNA. An analogous mechanism of regulation for some r-proteins was reported in yeast (Team and Rosbash 1983; Dabeva et al. 1986). The precise role of the final product in this regulatory process is still under investigation.

The persistence of the injected L1 gene and its expression provide the possibility of further studies by injection of mutagenized genes to identify DNA sequences involved in regulation at the post-transcriptional and translational levels.

Materials and methods

Injection of fertilized eggs and oocytes

Artificially fertilized eggs, obtained from hormone-stimulated *Xenopus laevis* females and dejelled before fertilization, were placed on nylon nets in dechlorinated water and injected in the animal hemisphere within an hour with 10 nl of buffer or DNA solution delivered by a glass needle connected with a mechanically driven microsyringe. The fertilization protocol was as described by Rusconi and Schaffner (1981). Developing embryos were incubated in dechlorinated water plus 150 µg/ml of penicillin and streptomycin at 20°C. At the appropriate stage, 1 µCi of [³⁵S]methionine in 100 nl (New England Nuclear, sp. act. 800 Ci/mmol) was microinjected into the dorsal part of the embryo, which is impermeable to amino acids. After labeling, embryos were collected in groups of eight, washed, quickly frozen in dry ice, and stored at –70°C. Oocytes were microinjected in the nucleus according to Bozzoni et al. (1984).

Preparation of cell extracts

Each group of embryos was homogenized still frozen in 100 μ l of a sterile solution containing 10 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM MgCl₂, 5 mM DTT, and 300 U/ml of RNase inhibitor (Boehringer). Half of the sample was rapidly placed in an Eppendorf tube containing 350 μ l of a solution containing 1 mg/ml Proteinase K and 2% SDS for extraction of nucleic acid (Probst et al. 1979); the other half was acid-extracted for preparation of proteins (Pierandrei-Amaldi and Beccari 1980). When preparing polysomes, nuclei were pelleted at 2000 rpm for 5 min and cytoplasmic fractions were separated on 15–50% sucrose gradients as already described (Pierandrei-Amaldi et al. 1985a). The fractions corresponding to polysomes and mRNP were pooled and precipitated with 3 volumes of ethanol.

Extraction and analysis of DNA and RNA

Nucleic acids from embryos, oocytes, and gradient fractions were extracted with proteinase K/phenol/chloroform according to Probst et al. (1979). An aliquot of total nucleic acid was used for DNA analysis by Southern blot either undigested or after digestion with *Bam*HI. Poly(A)⁺ RNA was obtained by oligo(dT)-cellulose chromatography and was analyzed by Northern blotting. Filters were hybridized to ³²P-labeled L1 and L14 cDNA single-stranded probes as previously described (Pierandrei-Amaldi et al. 1985a).

S1 analysis

S1 nuclease mapping was carried out according to the procedure described by Bozzoni et al. (1984). The probe utilized is an 813-bp long *Xba*I–*Pvu*II fragment that includes 633 bp of the L1 gene extending from the *Xba*I site of intron 3 to the *Pst*I site of exon 2, plus 180 bp coming from the *Pst*I–*Pvu*II sequence of the pSP-65 plasmid in which the fragment has been cloned utilizing the *Xba*I and *Pst*I sites in the polylinker region. The fragment was 5'-end-labeled at the *Xba*I terminus with polynucleotide kinase and annealed in 80% formamide, 0.4 M NaCl, 40 mM MOPS (3-*N*-morpholine acid) (pH 6.7), and 1 mM EDTA together with total RNA. The annealing was allowed to proceed for 12 hr at 50°C. The S1 reaction was performed as already described (Bozzoni et al. 1982), and the products were analyzed on a 6% acrylamide–urea gel.

Protein analysis

Proteins were analyzed on two-dimensional acrylamide gels as previously described (Pierandrei-Amaldi et al. 1982).

Densitometric analysis

X-ray films of Southern, Northern, and two-dimensional protein gels were quantitated by analysis with a LKB Ultrosan XL laser densitometer.

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