EXPRESSION OF RIBOSOMAL PROTEIN GENES IN *XENOPUS* DEVELOPMENT

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INTRODUCTION

Ribosome production is one of the major projects of developmental systems, such as *Xenopus* oocytes and embryos, which thus are particularly suitable for the investigation of the regulation of ribosome biogenesis. A typical feature of the embryo is its utilization of maternal stored material accumulated during the oogenesis. In particular the *Xenopus* embryo is completely dependent on maternal gene products until the 'midblastula transition' (4000 - 8000 cells); at this stage transcription is activated and the embryo begins to use the products of its own genes (Newport and Kirschner, 1982). The stored material includes, beside proteins and mRNAs, a huge amount of ribosomes (10^{12} per oocyte) accumulated as 80S particles, which are sufficient to support protein synthesis for a considerable part of embryogenesis; when they become limited the embryo begins making new ribosomes. We were interested in studying, during this period of development, the regulation of expression of the protein component of the ribosomes, the numerous ribosomal proteins (r-proteins), whose coordinated production implies a fine regulation. A coregulation with ribosomal RNAs, with which they are functionally related, could also be expected.

EXPERIMENTAL SYSTEM AND APPROACHES

The information we have obtained on this problem came at first from the analysis of the expression of r-protein genes in developing oocytes and embryos. These studies have been carried out at various regulatory levels: transcription of r-protein genes, maturation and accumulation of their transcripts, synthesis and stability of the r-proteins (reviewed in Amaldi et al., 1989). As for the influence of rRNA genes on the expression of r-protein genes we had some answers from the anucleolate embryo, one of the few mutants available in Xenopus (Elsdale et al., 1958). This homozygous mutant, which carries a deletion of the rRNA gene cluster, can survive up to the tadpole stage using maternal ribosomes, which allow it to pass through the developmental period concerned with the production of new ribosomes. The third approach we have been using consists of the introduction of molecules (cloned genes, proteins and antibodies) in oocytes and embryos by microinjection, thus interfering with the normal pattern of expression of r-protein genes.

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REGULATION AT POST-TRANSCRIPTIONAL AND TRANSLATIONAL LEVELS

The data obtained from the various experimental approaches indicated that the expression of r-protein genes in *Xenopus* development involves a regulation at the level of stability of the r-protein mRNA, apparently affected by the accumulation of unutilized r-proteins (Pierandrei-Amaldi et al., 1985). A more detailed analysis of the gene for r-protein L1 has shown that the production of L1mRNA is controlled by the L1 protein itself which interferes with the correct processing of the transcripts (Bozzoni et al., 1984; Caffarelli et al., 1987; Pierandrei-Amaldi et al., 1988). Another typical feature of these genes is that they are also controlled at the level of translation. Supporting evidence for this comes from the observation that during development the production and accumulation of r-protein mRNA (rp-mRNA) is uncoupled from its utilization (Pierandrei-Amaldi et al., 1982; Baum and Wormington, 1985). In fact rp-mRNA starts to be synthesized and accumulated at the blastula stage, when many genes become transcriptionally activated. This mRNA remains for several hours mostly in mRNPs, and is mobilized onto polysomes around the tailbud stage. The mobilization, which leads to the synthesis of new r-proteins and is concomitant with a significant increase of rRNA (Brown and Littna, 1964), appears to respond to a shortage of ribosomes when the maternal store has been used up (Pierandrei-Amaldi et al., 1985). A similar delay in the untilization of rp-mRNA was observed also during oogenesis (Cardinali et al., 1987). It could be expected that this translational control could be autogenously regulated as in *E. coli* where r-proteins, if in excess relative to the rRNA, prevent further translation of their mRNA (Nomura et al., 1984). In order to test this hypothesis, we injected an excess of purified *Xenopus* r-proteins in *Xenopus* oocytes to see if they had any effect on the endogenous synthesis of r-proteins. Similar experiments were carried out by adding excess proteins to an *in vitro* system programmed with mRNA coding for *Xenopus* r-proteins. No inhibition of r-protein synthesis was obtained in these experiments suggesting that the translation of rp-mRNA in *Xenopus* is not feed-back regulated (Pierandrei-Amaldi et al., 1985b). This conclusion was even more strongly supported by experiments in anucleolate embryos, where it has been observed that the absence of rRNA genes does not interfere with the synthesis of rp-mRNA nor with the synthesis of r-proteins; however newly synthesized r-proteins, which do not find rRNA to assemble with, are unstable and are degraded (Pierandrei-Amaldi et al., 1985). A similar behavior was reported in this mutant for 5S RNA (Miller, 1974), thus indicating that the absence of rRNA has no effect on the synthesis of the other ribosomal components but is crucial for their stability.

EXPERIMENTAL ANALYSIS OF TRANSLATIONAL CONTROL

As mentioned above, the distribution of rp-mRNA between the translationally active polysomes and inactive mRNPs changes specifically during development: the percentage of rp-mRNA loaded on polysomes increases progressively from stage 26 to stage 30, when stored ribosomes become limited. A similar pattern is observed in the anucleolate mutant in the early period of r-protein synthesis; however when at later stages the maternal store is finished, and no ribosomes can by synthesized, the rp-mRNA available is completely recruited onto polysomes (Pierandrei-Amaldi et al., 1985). The temporal relationship between shortage of ribosomes and rp-mRNA recruitment suggested to us the idea that the amount of ribosomes available in the cell might interfere with the efficiency of translation of rp-mRNA and consequently with the production of new ribosomes. To test this possibility we experimentally modified the amount of available ribosomes in developing embryos: an increase was obtained by microinjection of purified ribosomes into fertilized eggs, a decrease was induced by treatment with a drug which reduces the amount of 80S and ribosomal subunits. The effect of this

manipulation on the partition of rp-mRNA between polysomes and mRNA was analyzed in developing embryos: an inverse relationship between the amount of ribosomes and rp-mRNA loading on polysomes was observed (Pierandrei-Amaldi et al., submitted). Although these results suggest that the amount of available ribosomes signals the need for new ones, as also indicated by experiments carried out in *Drosophila* (Schmidt et al., 1985), we do not know how this is achieved. Some particular structure in the rp-mRNA, perhaps together with specific factor(s), could be responsible for the observed control. Structural elements present at the 5' untranslated region of the mRNA for r-protein S19, also shared by other rp-mRNAs, were identified as able to confer the characteristic translational pattern during embryo development to the mRNA carrying them (Mariottini et al., this volume). On the other hand it was reported that the dissociation from polysomes and deadenylation of mRNA for r-protein L1 in maturing oocytes in mediated by the 3' portion of mRNA (Hyman and Wormington, 1988).

An interesting feature of the translational control emerged from experiments of gene dosage increase obtained in developing embryos by microinjection of the cloned gene for r-protein L1 in fertilized eggs. This manipulation induced in the embryo a tenfold increase of the corresponding mature mRNA which, although present in a higher amount, maintained a relative distribution between polysomes and mRNPs similar to controls and typical of the developmental stage. This indicates that the percent, rather than the absolute amount, of rp-mRNA to be loaded on polysomes is regulated (Pierandrei-Amaldi et al., 1988). These results suggest the possibility that some factor(s), which may act in a positive or negative way on the rp-mRNA translation, could be involved. Considering that the various rp-mRNAs share common structural features at their 5' end, one can speculate on the implication of a particular factor(s) able to recognize similar structures and interact with this class of mRNAs for a coordinated translational regulation.

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