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Identification of *Xenopus laevis* mRNAs with homology to repetitive sequences

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ABSTRACT

Hybrid selection translation experiments have been carried out with genomic and cDNA relatives of two repetitive sequence families. On the basis of the in vitro translation products detected, it was found that transcripts complementary to these repeats are linked to several different mature mRNAs in stage 40 embryos of Xenopus laevis. One repeat hybridizes to mRNAs that direct the synthesis of 17 proteins. The second is present on mRNAs coding for 3 proteins. By estimating the abundance of these proteins among the translation products of total embryonic mRNA, it is inferred that all of the repeat bearing mRNAs are rare, less than one in 20'000 mRNA molecules.

INTRODUCTION

A major fraction of the eukariotic genome consists of repetitive sequences that are interspersed among single copy DNA regions. These interspersed repeats can be grouped into families of related sequences on the basis of sequence homology. Members of various dispersed repeat families in organisms such sea urchins (1-3), Xenopus laevis (4-6), and man (7) have been cloned and characterized. The fact that copies of such repeats are disseminated throughout the genome implies that homologous sequences lie in the vicinity of genes at many different genomic loci. It is therefore possible that repetitive sequences could be regulatory elements involved in the coordinate expression of functionally related genes (8). Although there is as yet no direct proof for this hypothesis, a certain amount of evidence in support of it has been provided by results indicating that interspersed repeats may be transcribed in a cell specific or developmentally regulated manner (9).

Repetitive sequences are extensively represented in cellular RNA. Interspersed repetitive sequence transcripts exist for example in the nuclear RNA of sea urchin embryos (10), rat ascites cells (11), and Hela cells (12). Similar transcripts have been detected in the maternal RNA populations of sea urchin (13) and Xenopus laevis eggs (14, 15). The representation of specific repeats in such transcripts has been extensively analyzed in the sea urchin (16-18). It was found that, for a given repeat, both complements are generally represented and that both the size and concentration of the transcripts vary as a function of cell type. On the basis of these observations it has been proposed that repetitive sequences in presumed mRNA precursors in nuclear RNA of somatic cells or in maternal RNA of eggs could play a regulatory role in the production of sets of mRNA molecules (19, 20).

Results from several groups also suggest that repeat transcripts are present on mature mRNAs. Initial evidence was based on the hybridization kinetics of mRNA with an excess of genomic DNA (21, 22). It was subsequently shown that specific cloned genomic repeats hybridize to polyadenylated cytoplasmic RNA (23-26). Others have identified repeats in cDNA clones (27-29).

We have been working with repetitive sequences that are transcribed in the embryo of Xenopus laevis. A genomic clone has previously been shown to contain interspersed repeats that are homologous to a large number of different transcripts in polyribosomal RNA of stage 40 embryos (4). Here we present evidence that these repetitive sequence transcripts are covalently linked to mature mRNAs that are translatable in vitro. On the basis of their homology to two different repetitive sequences, these repeat bearing mRNAs can be divided into two sets of structurally related molecules.

MATERIALS AND METHODS

Animals

Ovulation and mating were induced in Xenopus laevis by injection of chorionic gonadotropin (30). Embryos were grown in aerated water at 20-24^{o}C until they reached stage 40 (31).

Preparation of DNA and RNA

The isolation of clones, extraction of plasmid DNA, and purification of DNA fragments have been described previously (4, 32). Cytoplasmic RNA was prepared from stage 40 embryos by a phenol/chloroform extraction (33) of the postmitochondrial supernatant obtained as described (34). RNA was precipitated several times with ethanol, and twice with 2M LiCl overnight at 4°C. Enrichment of polyadenylated RNA was achieved by oligo (dT)-cellulose chromatography (35).

Blot hybridization

RNA was denaturated with glyoxal (36), fractionated by electrophoresis in 1% agarose gels, transferred to DBM paper (37) and hybridized with nick translated probes as described previously (4).

Hybridization selection

Squares of ABM (aminobenzyloxymethyl) paper (Schleicher and Schuell) with an area of about 1.5 cm^2 were diazotized (37), and linearized plasmid DNA or purified DNA fragments were bound to the DBM (diazobenzyloxymethyl) paper (38). The DNA bound squares were cut into four pieces and stored at 4°C in 50% formamide, 0.6 M NaCl, 4 mM EDTA, 20 mM PIPES (1.4-piperazinediethanesulfonic acid) at pH 6.4, and 0.1% SDS. Hybridization of DNA paper, carrying up to 100 µg of plasmid DNA or 20-30 µg of purified DNA fragments, with cytoplasmic RNA from stage 40 embryos was done by a method modified from that described elsewhere (39). Prior to hybridization, DNA paper was prehybridized for 5 hours at 40°C in hybridization buffer (50% formamide, 0.6 m NaCl, 4 mM EDTA, 20 mM PIPES (pH 6.4), 0.1% SDS, 250 µg/ml poly(rA), and 250 µg/ml calf liver tRNA), and heated for 30 minutes at 65°C in 99% formamide, 10 mM Tris-HCl (pH 7.8). Cytoplasmic RNA was precipitated with 0.3 M Na Acetate (pH 5) and 3 volumes of ethanol, and resuspended directly in hybridization buffer at a concentration of 4 to 5 mg/ml. The RNA in hybridization buffer was then heated to 70°C for 15 minutes and cooled before the DNA paper squares were added. Hybridization was allowed to proceed for 16-20 hours at 40°C. After hybridization, washes of the DNA paper and elution of hybridized RNA were done essentially as described (39). Hybrid

selected RNA from two consecutive hybridization selections was pooled, precipitated twice with 0.3 M Na Acetate (pH 5) and 3 volumes of ethanol, and resuspended in 1 or 2 µl of double distilled water.

Cell-free translation

Micrococcal nuclease treated rabbit reticulocyte lysate was purchased from Amersham. Hybrid selected RNA was heated to 100°C for 1 minute prior to translation. RNA samples were translated in a 20 µl reaction that contained 3-4 µCi/µl of L-[^{35}S]methionine (Amersham, > 800 Ci/mmol) and 3-4 µCi/µl of a tritiated amino acid mixture (Amersham, TRK 550). Translation assays were generally supplemented with 5 mM cAMP, 1 U/µl of human placental ribonuclease inhibitor (Bethesda Research Laboratory) and 0.1-0.2 µg/µl of calf liver tRNA (Boehringer Mannheim). After translation, samples were treated with 0.2 µg/µl of RNAse for 10 minutes at 37°C.

Gel electrophoresis of translation products

SDS-polyacrylamide slab gels for the electrophoresis of proteins (40, 41) consisted of a 15% acrylamide resolving gel and a 5% stacking gel. Two dimensional gel electrophoresis was done essentially as described (42). Ampholine concentrations used in the isoelectric focusing gel were 1.1% pH 5-7 (LKB), 1.1% pH 5-7 (SERVA) and 0.8% pH 3.5-10 (LKB). The second dimension consisted of a 15% acrylamide resolving gel and a 5% stacking gel. After electrophoresis gels were processed for fluorography with EN^{3}HANCE (New England Nuclear) and exposed to preflashed Kodak X-Omat AR-5 film. For quantitation, spots on two dimensional gels were cut out, rehydrated in 100 µl of water for 3 hours, treated with SOLUENE 350 (Packard) for 3 hours at 55°C, and counted in a scintillator containing 5 g PPO and 0.5 g Bis-MSB per liter of toluene.

RESULTS

Repetitive sequence transcripts homologous to the genomic clone X132

We have previously described a genomic clone from X.laevis called X132 (4). X132 contains both unique and repetitive se-

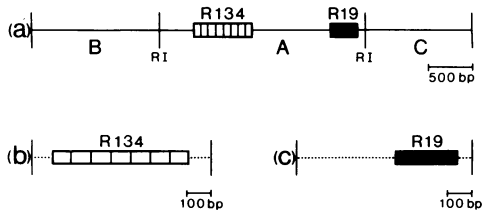

Fig. 1. Repetitive sequence homology between genomic clone X132 and cDNA clones C134 and C19. (a), partial restriction map of X132 showing the positions of the EcoRI (RI) subfragments A, B and C, and of the repeats R134 (open boxes: 8 subunits) and R19 (solid box); (b), clone C134 consisting essentially of 7 subunits of an R134 repeat (open boxes); (c), clone C19 containing an R19 repeat (solid box). Dotted lines in (b) and (c) indicate cDNA sequences showing no homology to X132.

quences. Two different repeats, referred to here as R134 and R19, have been defined and characterized in X132. R134 consists of eight tandemly repeated subunits of 77 to 79 base pairs and is present in approximately 100'000 dispersed copies per genome (4). R19 is a stretch of about 300 base pairs that is reiterated at approximately 2'200 sites in the genome (32). The localization of R134 and R19 on clone X132 is indicated on the partial restriction map shown in Figure 1a. Digestion of X132 with the restriction enzyme EcoRI divides it into three subfragments, A (2.27 kb), B (1.4 kb) and C (1.2 kb). Fragment B consists exclusively of unique sequences. Both A and C fragments contain repeats but only the R134 and R19 elements in fragment A have been characterized in detail and sequenced.

Transcripts complementary to the unique sequence in fragment B and to the repeats in fragment A have been detected in polyribosomal RNA of stage 40 X.laevis embryos (4). The unique sequence hybridizes to a single mRNA species. The repetitive sequences in fragment A, however, hybridize to many distinct transcripts of different sizes. Most of these transcripts are polyadenylated. Figure 2 shows the result of an experiment in which poly(A)$^-$ and poly(A)$^+$ fractions of cytoplasmic RNA were subjected to electrophoresis in an agarose gel, transferred to DBM paper, and hybridized with [^{32}P] labelled fragment A. The complex profile of the

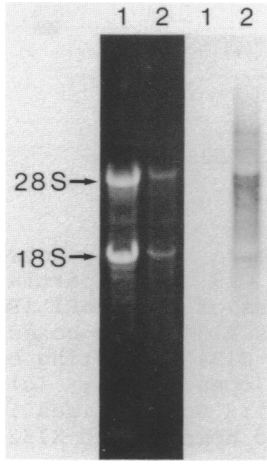

Fig. 2. Northern blot analysis of transcripts complementary to fragment A of X132 in poly(A)$^+$ and poly(A)$^-$ cytoplasmic RNA of stage 40 embryos. Cytoplasmic RNA was fractionated by oligo (dt)-cellulose chromatography. 15 µg of poly(A)$^-$ RNA (lane 1) and 5 µg of poly(A)$^+$ RNA (lane 2) were denatured with glyoxal, fractionated by electrophoresis in a 1% agarose gel, transferred to DBM paper and hybridized with nick translated fragment A of X132. The ethidium bromide stained gel is shown on the left and the autoradiograph of the hybridized filter on the right. Positions of 28S and 18S ribosomal RNA are indicated by arrows.

hybridization to transcripts that are found predominantly in the poly(A)$^+$ cytoplasmic RNA suggests that the repeats in fragment A are homologous to a large number of different mRNA molecules.

In an earlier study we tried to identify these presumptive repeat carrying mRNA molecules by isolating cDNA clones homologous to fragment A of X132. Two representative cDNA clones have been described elsewhere. Clone C134 (43) consists essentially of seven tandemly repeated subunits homologous to those of the R134 sequence (Figure 1b), and the clone C19 carries adjacent to the 3' end of an open reading frame a sequence homologous to R19 (Figure 1c), (32). These cDNA clones can not correspond to complete mRNAs, since they contain neither a poly(A) tail nor a potential polyadenylation signal for example. Hence, it was not possible to decide unequivocally whether they were derived from mature translatable mRNAs. In order to find out whether R134 and

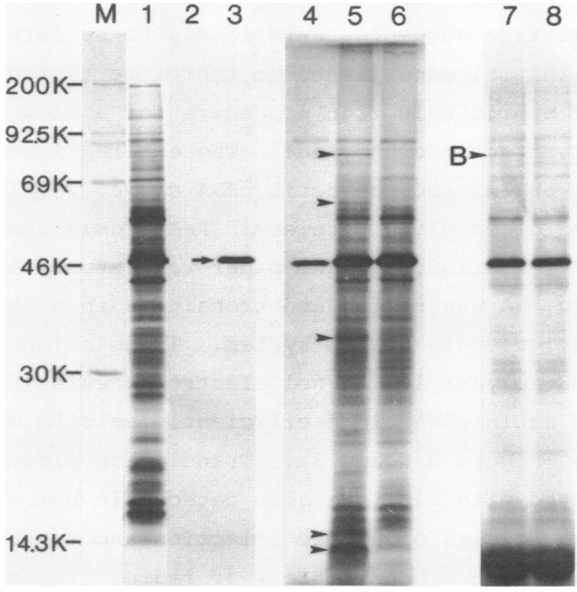

Fig. 3. Hybrid selection translation experiment carried out with the genomic clone X132. DNAs were bound to DBM paper and hybridized with cytoplasmic RNA from stage 40 embryos. Hybridized RNA was eluted and translated in a rabbit reticulocyte lysate system. Translation products were fractionated by electrophoresis in a 15% polyacrylamide-SDS gel. Lane M, marker proteins of indicated molecular weights (in kilodaltons). Translation products of the following RNAs were analyzed: lane 1, total embryo mRNA; lanes 2 and 4, RNA endogenous to system in absence of added mRNA; lane 3, hybrid selected actin mRNA; lane 5, RNA selected with X132; lanes 6 and 8, RNA selected with pBR322; lane 7, RNA selected with fragment B of X132. Arrows indicate actin (lane 3), X132 specific proteins (lane 5), and fragment B specific protein (lane 7). Lanes 2 to 8 were exposed for 10 days. Proteins were labelled with [^{35}S]methionine plus [^{3}H] amino acids in lanes 1 and 4-8, but with only [^{3}H] amino acids in lanes 2 and 3 in order to eliminate the endogenous [^{35}S]-labelled band comigrating with actin (lane 4). Aliquots of hybrid selected RNA translated were in samples 5-8 twenty times larger than in sample 3.

R19 repeats are indeed found on mRNAs we carried out hybrid selection translation experiments with these sequences.

Hybrid selection translation experiments carried out with X132

The intensity of the hybridization observed in Northern blotting experiments and the number of positive clones detected

in a cDNA library (32) had suggested to us that mRNAs carrying the X132 repetitive sequences were likely to be rare in stage 40 embryos. In initial experiments we therefore tested the efficiency of our hybrid selection procedure for a known mRNA by using a X.laevis actin cDNA clone. The entire clone X132 and two control DNAs, pBR322 and the actin cDNA clone, were bound to DBM paper and hybridized with cytoplasmic RNA from stage 40 embryos. Following hybridization, the DBM paper was extensively washed and the hybridized RNA was eluted and translated in a nuclease treated rabbit reticulocyte lysate system. Translation products were analyzed by SDS-polyacrylamide gel electrophoresis (Figure 3). As expected, the actin cDNA clone efficiently selects mRNA that codes for actin (Figure 3, lane 3). Translation products specific for RNA selected with X132 are also detectable but only if RNA is pooled from two cycles of hybrid selection and translated in one assay, and when high concentrations of radioactive amino acids are used to increase the specific activity of the proteins synthesized. Several X132 specific bands are visible over a background pattern that is identical for both the X132 and pBR322 hybrid selected translation products (Figure 3, lanes 5 and 6). When a similar hybrid selection translation experiment is performed with the purified subfragment B, which consists of unique sequences, only one of the proteins specific to the X132 selected RNA is detected (Figure 3, lanes 7 and 8). This protein has an estimated molecular weight of 78 kd and is probably coded for by the mRNA that is detected when polysomal RNA is probed with fragment B in Northern blot experiments (4). This protein is however never detected when a hybrid selection translation experiment is carried out with any other region of clone X132. The mRNA which codes for the 78 kd protein can therefore not carry a sequence that is homologous to any of the X132 repetitive elements. The other proteins specific to the X132 selected material, however, could be coded for by mRNAs selected with one of the X132 repetitive elements.

The background visible in Figure 3, lanes 5-8, resembles the band pattern obtained when total stage 40 embryo mRNA is

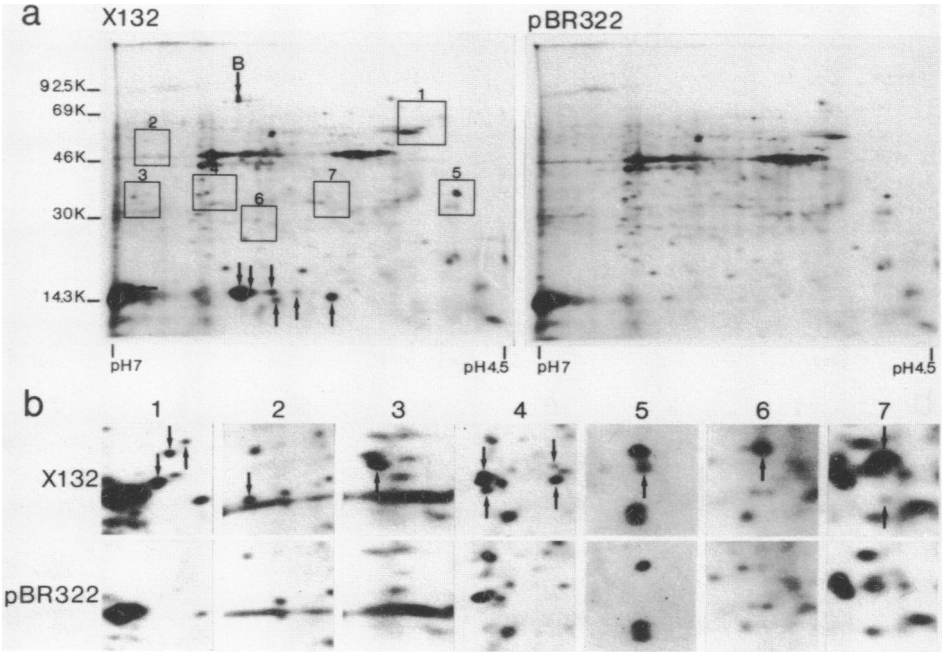

Fig. 4. Translation products of mRNA selected with the genomic clone X132. (a), two dimensional gel electrophoresis of the translation products of RNA selected with X132 (left) and pBR322 (right). The pH range of the first dimension and positions of molecular weight marker proteins in the second dimension are indicated. Boxes numbered 1-7 enclose regions of such gels shown after longer exposures in (b).
Arrows indicate proteins specific to the X132 gels. The protein labelled B is coded for by a mRNA that is selected by the unique sequence in fragment B of X132.

translated (Figure 3, lane 1). It must therefore result from mRNA that sticks unspecifically to the DNA paper, and is not adequately removed during the washes. Despite extensive washes of the DNA paper, we were not able to reduce this background. Although the background is not problematic when an abundant mRNA such as that coding for actin is selected and translated, it becomes a serious limitation of the method when used to detect the translation products of rare and inefficiently selected or poorly translated mRNAs. Such translation products could go unnoticed if they are covered by the background.

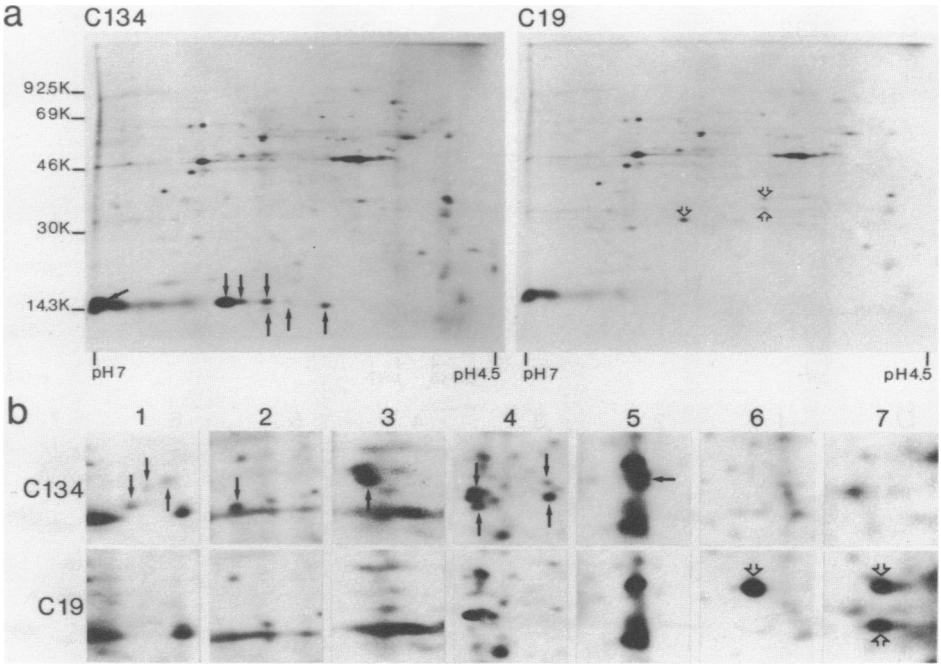

Fig. 5. Translation products of mRNA selected with cDNA clones C134 and C19. (a), two dimensional gel electrophoresis of the translation products of RNA selected with C134 (left) and C19 (right). The pH range of the first dimension and positions of molecular weight marker proteins in the second dimension are indicated. (b), details from such gels after longer exposures. Positions of details 1-7 are the same as in Figure 4. Solid arrows, proteins specific to C134 gels; open arrows, proteins specific to C19 gels. Enrichment over background of arrowed proteins in details 6 and 7 (particularly lower protein in detail 7) is greater than in corresponding details in Figure 4.

To overcome this problem we have tried to reduce the background by fractionating the translation products on two dimensional gels. When we compare the two dimensional gels for the X132 and pBR322 hybrid selection translation samples, up to 21 proteins can be detected that are present on the X132 gel but absent on the pBR322 gel. In Figure 4a short exposures of such gels are shown. Eight specific proteins can be detected, one of these being the one coded for by a mRNA that hybridizes to the unique sequence in fragment B of X132. After longer exposures

(Figure 4b) up to 13 additional protein spots can be detected. The detection of such a large number of mRNAs with sequence homology to clone X132 is consistent with the hybridization pattern obtained when fragment A is used as a probe in Northern blotting experiments (Figure 2).

Hybrid selection translation experiments carried out with the cDNA clones C134 and C19

cDNA clones C134 and C19 each carry a sequence that is homologous to a repetitive element found in subfragment A of the genomic clone X132. It seemed likely that one or both of these repetitive sequences are the ones that hybridize to the mRNAs we detected above. We therefore carried out a hybrid selection translation experiment with the purified inserts of clones C134 and C19. The translation products were fractionated on two dimensional gels. Short exposure of these gels (Figure 5a) clearly shows that each cDNA clone selects some of the mRNAs detected in experiments carried out with the genomic X132 clone. After longer exposures of such gels (Figure 5b) it is seen that of the 21 proteins detected on the X132 gel, 17 are also detected with cDNA clone C134 and 3 are detected with cDNA clone C19. The 78 kd protein that is coded for by a mRNA selected with the unique sequence (fragment B) of X132 is detected with neither of the two cDNA clones. Since the only sequences that are shared by the genomic clone X132 and the cDNA clones C134 and C19 are the repetitive sequences R134 and R19, respectively, it follows that each of these repeats is present on a set of mRNAs coding for different proteins. The mRNA set on which the R134 repeat is found codes for at least 17 different proteins which range in molecular weight from 14 to 70 kd. The mRNA set on which the R19 repeat is found codes for at least 3 different proteins of molecular weights 30, 33 and 35 kd.

Abundance of the repetitive sequence bearing mRNAs

Most of the mRNAs that carry R134 and R19 repetitive elements are selected with a low efficiency. This suggests that they are rare in the mRNA population of stage 40 embryos. In order to obtain an estimation of their abundance, we translated total stage

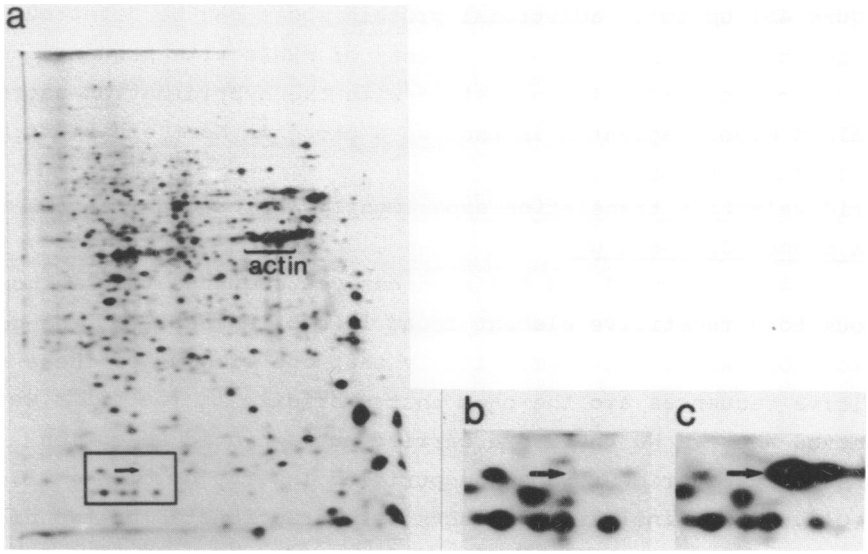

Fig. 6. Abundance of the mRNA selected most efficiently with the R134 repetitive sequence. (a), two dimensional gel electrophoresis of the translation products of stage 40 embryo mRNA. Boxed region is shown enlarged in (b). (c), corresponding region of a two dimensional gel on which were fractionated the translation products of RNA selected with X132. Arrows indicate the protein that is enriched most efficiently by the hybrid selection translation procedure. This protein was cut out of a gel such as the one shown in(a) and the amount of radioactivity present in it was counted. Taking the amount of radioactivity present in actin as a reference it was estimated that the arrowed protein accounts for approximately 0.005% of the translation products of embryonic mRNA.

40 embryo mRNA and fractionated the proteins synthesized on a two dimensional gel. We then compared this gel with those on which the translation products of RNA selected with X132 were fractionated, and tried to identify the proteins that were enriched by the hybrid selection translation experiments. Most of these proteins are not detectable among the translation products of stage 40 mRNA, confirming that the mRNAs which code for them are rare. The protein detected most efficiently with the R134 repeat is however weakly visible (Figure 6).

By counting the radioactivity present in spots cut out of a gel such as the one shown in Figure 6, it was estimated that the

incorporation of [^{35}S]-methionine into this protein is at most 0.1% of that incorporated into actin. A densitometric scanning of a film (Figure 3, lane 1) indicates that actin accounts for 5% of the total protein synthesized by stage 40 mRNA. The protein that is enriched most efficiently by the hybrid selection translation experiments described above therefore represents approximately 0.005% of the translation products of embryonic mRNA. If one assumes that translational activity in the in vitro translation system reflects accurately the abundance of a mRNA, the mRNA selected most efficiently with the Rl34 repetitive sequence would thus represent 0.005% of the mRNA population in stage 40 embryos, or one in 20'000 mRNA molecules. This value corresponds closely to a previous estimation based on the number of positive cDNA clones found in a cDNA library prepared from stage 40 polysomal RNA (32). Since the other repetitive sequence bearing mRNAs are selected with a considerably lower efficiency, they are probably significantly rarer. A frequency of less than one in 20'000 mRNAs implies that the detected mRNAs belong to the high complexity class of rare embryonic mRNAs (44). It should be pointed out however that the low abundance we have calculated is based on the mRNA content of the heterogeneous cell population of the entire embryo and the possibility that the detected mRNAs could be more abundant in defined tissues can consequently not be excluded.

DISCUSSION

We have performed hybrid selection translation experiments to demonstrate that sequences complementary to two repetitive elements, Rl34 and Rl9, are present on mRNA molecules in stage 40 X.laevis embryos. The Rl34 repeat, which is shared by the genomic clone Xl32 and the cDNA clone Cl34, was found to hybridize to a set of mRNAs that are translatable into 17 different polypeptides. Rl9, which is also present on Xl32 and in cDNA clone Cl9, selects a different set of mRNAs that code for 3 proteins. It is possible that more translation products exist but have escaped detection because they are present in small amounts.

Several additional proteins have indeed been detected at low levels. Moreover, we have analyzed only acidic translation products. The number of mRNAs identified is therefore likely to be underestimated. It should be pointed out that we have identified the mRNAs on the basis of an in vitro translation assay. Clearly the identification of the in vitro translation products would provide stronger evidence. The specific antibodies required to accomplish this are however not yet available.

All of the mRNAs are selected with a low efficiency. This is mainly due to their low abundance, which we have estimated to be less than 0.005% of the stage 40 embryo mRNA population. A second factor which may contribute to inefficient selection is sequence divergence between the cloned repeats and the homologous sequences on the mRNAs. There is some evidence that this may be the case, particularly for the mRNAs that carry a copy of the R19 sequence. Under the same conditions, the C19 cDNA clone is more effective in selecting the mRNAs than the genomic X132 clone is (compare figures 4 and 5). Although this is expected for the mRNA from which the cDNA was derived, it suggests that the repeats on the other mRNAs are more homologous to the cDNA repeat than to the genomic relative. Moreover, the genomic X132 clone selects the R19 homologous mRNAs more efficiently when the stringency of the hybrid selection procedure is reduced (data not shown), indicating that sequence divergence may be a limiting factor for the amount and number of selected mRNAs. Nucleotide sequences of the repeats in the genomic clone X132 and in the cDNA clones C134 and C19 have been presented and compared elsewhere (4, 32, 43).

In other systems, repetitive sequence transcripts have been identified at the 5' (23) and at the 3' end of mRNA molecules (26-29). We suspect that the mRNAs we have identified carry the repeat in the 3' untranslated trailer region. Sequence analysis of the R19 and R134 repeats in the C19 and C134 cDNA clones (32, 43) has indicated that ATG translation initiation codons are shortly followed by translation arrest codons in all reading frames of the repetitive elements. This feature is generally in-

consistent with the organization of both 5' untranslated leader sequences (45) and coding regions of mRNAs. The repeat on the C19 cDNA is furthermore found adjacent to the 3' end of an open reading frame, suggesting that it is in the 3' untranslated trailer of the corresponding mRNA (32). It can not be excluded however that the C19 and C134 cDNA clones are not representative of all the detected mRNAs. We have therefore started to perform hybrid arrested translation experiments (46). Preliminary results indicate that translation of the mRNA molecules is not inhibited when the repetitive elements are in hybrid conformation. This finding also suggests that the repeats are part of the 3' untranslated trailers.

Most of the translation products we detect differ both in size and isoelectric focusing point. Among the translation products of mRNAs that carry the R134 element there are nevertheless seven which, although they have different isoelectric focusing points, all have a molecular weight close to 14.5 kd. This apparent similarity may indicate that these proteins are coded for by a gene family similar to the one described in sea urchins (28). It is also possible however that they are unrelated gene products, or that some of them are simply modified forms of the same protein. Modifications affecting the isoelectric focusing point such as phosphorylation (47) and acetylation (48) are known to occur in the rabbit reticulocyte lysate system. Artefactual charge modifications may also occur during the storage of protein samples (42). Further studies are therefore required to determine whether the apparent similarity of the seven small proteins is significant.

One may wonder whether the repeat transcripts we have detected could be of maternal origin, since in Xenopus laevis oocytes a major fraction of the cytoplasmic poly (A) RNA contains interspersed repetitive sequences (14). This maternal RNA however, in spite of its cytoplasmic localization, resembles nuclear RNA rather than mRNA and is thought to consist of non-translatable sequence (18). On the contrary the RNAs identified here have, on the basis of Northern blot and hybrid selection translation expe-

riments, the properties of mRNA. It is therefore unlikely that they represent remnants of maternal interspersed poly(A) RNA. We have recently confirmed this for part of the mRNAs by performing hybrid selection translation experiments with RNA from mature oocytes. Preliminary results (Reith et al., unpublished data) show that the R19 homologous mRNAs, and the R134 homologous mRNAs that code for the major group of small 14.5 kd proteins, are not present in the oocyte. Consequently, at least part of the mRNAs must be synthesized during embryogenesis.

It is tempting to speculate that the repetitive sequences found on mRNA molecules could function as coordinating regulatory elements. If this is the case one would expect the mRNAs that carry the same repeat to behave as a set of functionally related molecules. Their presence in specific developmental stages or different tissues might be expected to be coordinately regulated. In future experiments we will therefore search for the mRNA molecules carrying the R134 or R19 repeat in embryos of different stages and in various adult tissues. Such studies should allow us to determine whether these two sets of structurally related mRNA molecules are transcribed from coordinately expressed genes.

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