Purification of a calcium dependent ribonuclease from *Xenopus laevis*

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ABSTRACT

We have purified a Ca^{2+} dependent ribonuclease from the oocytes of *Xenopus laevis*. Two properties of this ribonuclease set it apart from other known nucleases. First, Ca^{2+} was required for ribonuclease activity, and Mg^{2+} would not substitute. Second, the enzyme specifically degraded RNA and digestion of double or single stranded DNA was not observed. Ca^{2+} dependent ribonuclease activity of the purified 36-kDa protein was directly observed after renaturation of the protein following electrophoresis in an SDS-Laemmli gel. In addition, the enzyme was shown to have endoribonuclease activity at numerous sites. The Ca^{2+} dependence suggests that the ribonuclease activity may be modulated by changes in the level of intracellular Ca^{2+} and thereby provide a direct link to signal transduction systems.

INTRODUCTION

The intracellular degradation of RNA is a crucial step in the regulation of gene expression and plays a central role in determining the relative abundance of different RNAs. Almost every newly synthesized RNA molecule must be acted on by one or more RNases before becoming completely functional and each RNA is eventually degraded. Nevertheless, our understanding of the molecular mechanisms and molecules involved in the selective and general destruction of RNA lags far behind that for RNA synthesis. A number of RNases involved in the processing, turnover, and degradation of RNA in *E. coli* have been identified and characterized (1). In contrast, although the small extracellular eukaryotic RNases, e.g. pancreatic ribonuclease, are well studied, our knowledge of the eukaryotic RNases involved in intracellular RNA metabolism is fragmentary (2).

We discovered a Ca^{2+} dependent ribonuclease activity in extracts from *Xenopus* oocytes and proceeded to purify this enzyme because it had two interesting properties. The enzyme required Ca^{2+} for activity and was specific for RNA substrates. An intriguing possibility is that this ribonuclease could be regulated by the intracellular level of Ca^{2+} and thereby its activity would be directly coupled to signal transduction

pathways. We have succeeded in obtaining an essentially pure preparation of a 36-kDa protein with Ca^{2+} dependent ribonuclease activity. The purified Ca^{2+} dependent ribonuclease should provide a powerful tool in efforts to understand the relationship of this protein to cellular RNA metabolism.

MATERIALS AND METHODS

Ribonuclease activity assay and unit definition

RNase activity was assayed by the release of acid soluble radioactivity from a synthetic radioactively labeled RNA. Activity assays were done for 3 min at 22°C in a total volume of 25 μl in the following buffer: 20 mM sodium-HEPES (pH 7.6), 10 mM KCl, 1 mM CaCl_2 , 1 mM dithiothreitol, and 100 $\mu\text{g/ml}$ acetylated BSA. In addition to the radioactively labeled RNA, each reaction contained 100 ng of yeast ribosomal RNA. Different assays were done for dilutions of each sample and the volume of sample required to release 40% of the radioactivity into acid soluble form was used to calculate the number of units of RNase activity. One unit of RNase activity was defined as the amount of enzyme required to release one nmol of phosphate into acid soluble form in one minute under the conditions described above. To calculate the number of RNase activity units it was assumed that the unlabeled yeast ribosomal RNA was digested to the same extent as the radioactively labeled RNA. The radioactively labeled RNAs were produced by transcription of linearized plasmid DNA templates with phage T7 RNA polymerase in the presence of [α - ^{32}P] GTP using standard conditions (3, 4) and were purified before use by electrophoresis in 6% polyacrylamide gels containing 8 M urea. The most frequently used synthetic radioactively labeled RNA was 356 nucleotides long and was derived from the coding sequence of the *E. coli* β -galactosidase gene. To determine the amount of acid soluble radioactivity, the Ca^{2+} dependent ribonuclease reactions were stopped by the addition of EGTA to a final concentration of 8 mM, 1 μg of carrier yeast ribosomal RNA was added, and the reactions were adjusted to a final concentration of 5% trichloroacetic acid and 0.5% sodium pyrophosphate. After a 10 min incubation on ice, the reactions were centrifuged at full speed in an eppendorf centrifuge for 6 min, and 90% of the supernatant was counted in a scintillation counter. Using this assay, Ca^{2+} independent

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ribonuclease activity was not detected at any stage of the purification, including the crude extract.

The 91 nucleotide long RNAs used for mapping the cleavage sites were produced by phage T7 RNA polymerase transcription of plasmid DNA linearized with *PvuII*. The template plasmid was constructed by inserting a 47 bp fragment from the *E. coli* β -galactosidase gene (*RsaI* to *SspI*, positions 2481 to 2528 of GenBank locus ECOLAC) into the *EcoRV* site of the vector pSP70. The RNA was synthesized using T7 RNA polymerase, purified on a denaturing polyacrylamide gel, treated with alkaline phosphatase, and 5' end labeled using T4 polynucleotide kinase and [γ - 32 P] ATP or 3' end labeled using T4 RNA ligase and [5'- 32 P] pCp.

Purification of RNase X

The Ca^{2+} dependent ribonuclease, RNase X, was purified from whole oocytes of *Xenopus laevis* starting with an extract made using the following extract buffer: 30 mM sodium-HEPES (pH 7.5), 70 mM KCl, 1 mM DTT, 50 μ M EDTA, 50 μ M EGTA, and 10% glycerol (5). The whole oocyte extract (protein concentration ~ 6 mg/ml) was run over a phosphocellulose column (Whatman P-11, ~ 10 mg protein per ml resin) pre-cycled according to the manufacturers recommendations and equilibrated and run in extract buffer. The Ca^{2+} dependent ribonuclease activity was found in the flow through. The phosphocellulose flow through was then run over a heparin sepharose column (Pharmacia, ~ 45 mg protein per ml resin) which was also equilibrated and run in extract buffer. The Ca^{2+} dependent ribonuclease activity was again found in the flow through. Proteins which bound to the heparin sepharose column were eluted using a 1 M KCl step in extract buffer and the same column was then equilibrated in buffer A: 30 mM sodium-HEPES (pH 7), 50 mM KCl, 1 mM CaCl_2 , 1 mM DTT, and 10% glycerol. CaCl_2 was added to the heparin sepharose flow through to reach a final concentration of 1 mM CaCl_2 , and was loaded onto the re-equilibrated ~ 7 ml heparin-sepharose column. Although most of the proteins flowed through the column again, the Ca^{2+} dependent ribonuclease activity bound to the heparin-sepharose column in the presence of Ca^{2+} . After washing the column with 4 column volumes of buffer A, RNase X was eluted from the column using a 60 ml linear gradient from 50 mM to 1 M KCl in buffer A. Fractions containing RNase X activity were pooled and vacuum dialyzed against buffer B: 20 mM Tris-HCl (pH 8.8), 10% glycerol. A Mono Q column (Pharmacia HR5/5) attached to an HPLC (Hewlett-Packard 1050) was equilibrated in buffer B and 5 mg of acetylated BSA was loaded onto the column and then eluted with a linear gradient of 0 to 1 M KCl in buffer B. After two additional gradient runs to clear the column, the dialyzed fractions from the heparin sepharose column containing RNase X activity

were loaded onto the Mono Q column in buffer B. The Ca^{2+} dependent ribonuclease activity was eluted from the column using a 15 ml linear gradient of 0 to 0.5 M KCl in buffer B. RNase X eluted as a sharp peak at ~ 120 mM KCl and by choosing the appropriate fraction an essentially homogeneous preparation of the protein was obtained. At various stages in the preparation the samples were frozen in liquid nitrogen and stored at -70°C .

SDS-PAGE, gel staining, and protein concentrations

Protein concentrations were determined using the Bradford method (Bio-Rad) with BSA as the standard (6). Proteins were separated by electrophoresis on 11% SDS-polyacrylamide gels (7). Proteins were visualized by staining with Coomassie Brilliant Blue and by silver staining as described (8, 9).

Ribonuclease activity of proteins renatured in an SDS gel

Activity staining of SDS-Laemmli gels was carried out using an embedded radioactive RNA as a substrate for the renatured ribonuclease (10). Radioactive RNA (~ 2500 nucleotides long) was synthesized by phage T7 RNA polymerase transcription of a linearized plasmid DNA in the presence of [α - 32 P] GTP. The radioactive RNA ($\sim 2 \times 10^6$ cpm) was incorporated into the separating portion of an 11% SDS-Laemmli gel during polymerization. Protein samples were heated to 56°C for 10 min and electrophoresed in the gel. The gel (15 ml) was then washed 4 times for 30 min each at 22°C in 100 ml of 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 0.5 mM dithiothreitol. The gel was cut in half. Throughout the rest of the procedure one half of the gel was kept in the same buffer and the other half was transferred to a new buffer containing calcium: 50 mM Tris-HCl (pH 7.5), 1 mM CaCl_2 , 0.1 mM EDTA, and 0.5 mM dithiothreitol. The gel pieces were soaked for 12 hours at 4°C , the buffers were changed, and the gel pieces were soaked for 8 hours at 30°C . Ribonuclease activity was then assayed by autoradiography of the gel.

RESULTS

Discovery of a calcium dependent ribonuclease activity

A report that an RNA molecule was a component of the silkworm RNA polymerase III transcription machinery prompted us to look for a similar RNA involved in RNA polymerase III transcription in extracts from the oocytes of *Xenopus laevis* (11). Evidence for the importance of RNA in the silkworm system was first obtained by demonstrating the sensitivity of the *in vitro* transcription system to inactivation by micrococcal nuclease. Micrococcal nuclease digests both DNA and RNA, has an absolute requirement for Ca^{2+} , and its action can be stopped by EGTA-chelation of Ca^{2+} . We carried out experiments similar to those reported for the silkworm system and found that the

Table 1. Purification of RNase X

Purification step	Protein mg	Specific activity units/mg	Recovery %	Purification fold
1. Oocyte extract	352	16	100	1
2. Phosphocellulose FT	336	24	141	1.5
3. Heparin Sepharose FT	308	18	98	1.1
4. Heparin Sepharose (Ca^{2+})	5.5	522	50	33
5. Mono Q (peak fraction)	0.077	5710	8	360

addition of Ca^{2+} alone destroyed the ability of *Xenopus* whole oocyte extracts to transcribe RNA polymerase III genes. Micrococcal nuclease was not needed for the inactivation process.

Finding that RNA polymerase III transcriptional activity was inactivated in a Ca^{2+} dependent process, we looked for evidence of Ca^{2+} dependent RNase, DNase, and proteolytic activities in the extract. Electrophoresis of samples of the oocyte extract on SD-Laemmli gels both before and after incubation with Ca^{2+} showed the same pattern of protein bands after staining with Coomassie Blue. Using this rather insensitive assay, Ca^{2+} dependent proteolysis of specific proteins could easily go undetected, but the results showed that the vast majority of proteins were not extensively degraded. Addition of supercoiled DNA to the oocyte extract resulted in the rapid transformation of the supercoiled DNA to the relaxed closed circular form as a result of the action of DNA topoisomerases. The same closed circular DNA products were observed when Ca^{2+} was present during the incubation. Thus, at this level of analysis, there was no evidence for a Ca^{2+} dependent DNase activity. However, we did detect a Ca^{2+} dependent ribonuclease activity. The fate of a radioactively labeled RNA molecule added to the oocyte extract was drastically different when Ca^{2+} was included. In the absence of Ca^{2+} the RNA remained intact, but when Ca^{2+} was added the RNA was rapidly degraded. The rapid destruction of the endogenous ribosomal RNA was also observed when the extract was incubated with Ca^{2+} .

Chromatography of oocyte extracts on phosphocellulose resulted in the separation of the RNA polymerase III transcription factors, which bound to the column, from the Ca^{2+} dependent ribonuclease activity, which came out in the flow through. More detailed studies of the Ca^{2+} dependent inactivation of RNA polymerase III transcription suggest that RNA polymerase III transcription factors, in particular TFIIC, are inactivated via Ca^{2+} dependent proteolysis (12). The Ca^{2+} dependent RNase activity does not appear to be involved in the Ca^{2+} dependent inactivation of RNA polymerase III transcription observed in *Xenopus* oocyte extracts.

Purification of the Ca^{2+} dependent ribonuclease

Table 1 summarizes the purification of the Ca^{2+} dependent ribonuclease, RNase X, from *Xenopus* oocytes. Fig. 1 illustrates the proteins present at each of the purification steps. Ovaries were removed from female *Xenopus laevis* and the oocytes were released from the connective tissue by digestion with collagenase. After extensive washing of the intact oocytes, an extract was made by centrifuging the oocytes at high speed. The Ca^{2+} dependent RNase activity was easily measured in the whole oocyte extract (see Materials and Methods). There was no detectable RNase activity of the extract in the absence of Ca^{2+} . The oocyte extract was run over a phosphocellulose column and the Ca^{2+} dependent RNase activity was found in the flow through. We next took advantage of a remarkable property of the ribonuclease. We found that the affinity of the ribonuclease for heparin sepharose was dependent on the presence of Ca^{2+} . In the absence of Ca^{2+} the ribonuclease did not bind to heparin sepharose, but in the presence of Ca^{2+} the ribonuclease did bind. We ran the preparation over the same heparin sepharose column twice, first in the absence of Ca^{2+} and then in presence of Ca^{2+} . During the first run the Ca^{2+} dependent ribonuclease was found in the flow through, but in the second run it was bound to the column and then eluted with a linear gradient of KCl. Only

a few other proteins shared this property with RNase X and this was an extremely effective purification step.

The fractions containing Ca^{2+} dependent RNase activity which were eluted from the heparin sepharose column were vacuum dialyzed and further fractionated by ion-exchange chromatography on a Mono Q column. Fig. 2 shows the profile of proteins eluted from the Mono Q column using a biphasic linear KCl gradient. The peak of Ca^{2+} dependent RNase activity is indicated and corresponds to the absorbance peak at 3.6 ml elution volume. Fig. 3 shows the SDS-PAGE analysis of the fractions from the region of the Mono Q column containing the Ca^{2+} dependent ribonuclease activity. The pattern of proteins in this silver stained gel shows that a 36-kDa protein co-chromatographed with the Ca^{2+} dependent RNase activity. Electrophoresis of 3 μg of protein contained in the peak fraction in an SDS-Laemmli gel showed only a single 36-kDa species after staining of the gel with Coomassie Blue (data not shown, but see Fig. 1 lane i). Silver staining of the same gel revealed the presence of a number of contaminating species present at very low concentrations. An essentially homogenous preparation of the 36-kDa protein was obtained by selecting only the peak fraction and this was used for further characterization and analysis.

Calcium dependent ribonuclease activity of the 36-kDa protein renatured in an SDS gel

In order to directly demonstrate the Ca^{2+} dependent RNase activity of the 36-kDa protein we took advantage of the ability to detect RNase activity of proteins renatured in SDS gels (10).

Figure 1. SDS-PAGE analysis of RNase X during purification. Samples from each of the purification steps were electrophoresed in an 11% SDS-Laemmli gel and the gel was stained with Coomassie Blue. The total amount of protein and the units of Ca^{2+} dependent RNase activity loaded onto the gel for each of the purification steps were as follows: (1) whole oocyte extract, 65 μg , 1 unit; (2) phosphocellulose flow through, 65 μg , 1 unit; (3) heparin sepharose flow through, 65 μg , 1 unit; (4) heparin sepharose with Ca^{2+} , 15 μg , 7 units; (5) Mono Q, 1 μg , 5 units. The molecular weights of the proteins used as size markers in lane a are indicated. Purified T7 RNA polymerase (lane b) and *E. coli lac* repressor (lane d) were also included as markers. A mixture of these two proteins was electrophoresed in lane l (0.5 μg each), lane m (1 μg each), and lane n (2 μg each).

Figure 2. Fractionation of RNase X by Mono Q ion-exchange chromatography. The pooled fractions containing RNase X activity from the previous purification step were bound to a Mono Q column and eluted with a biphasic linear gradient of KCl (dashed line). The elution profile, measured as absorbance at 280 nm, is shown (solid line). Fractions (0.25 ml) were collected and assayed for RNase X. The peak of Ca^{2+} dependent ribonuclease activity is indicated by the arrow and corresponds to the absorbance peak observed on the elution profile.

Radioactive RNA was incorporated into the separating portion of an SDS-Laemmli gel during polymerization and provided the substrate for RNase activity following electrophoresis of protein samples, removal of the SDS, and renaturation of the proteins. RNase activity was observed as a clear area in an otherwise black autoradiograph of the gel. Fig. 4 shows a precise alignment between the Ca^{2+} dependent RNase activity and the position of the 36-kDa protein. A similar assay carried out using the fractions across the Mono Q column showed a direct correspondence between the amount and position of the 36-kDa protein in a given fraction and the Ca^{2+} dependent RNase activity observed. This assay conclusively demonstrates that the 36-kDa protein has Ca^{2+} dependent RNase activity. We propose to name this ribonuclease RNase X (X for *Xenopus*).

Properties of RNase X

Two very interesting properties of RNase X are first, that it is a ribonuclease which digests RNA but not DNA, and second, that it requires Ca^{2+} for activity. Digestion of either supercoiled plasmid DNA or single stranded phage M13 DNA was not observed when the DNAs were incubated with RNase X in the presence of Ca^{2+} (data not shown). We tested the ribonuclease activity of RNase X in the presence of other divalent ions using two assays. Using the release of acid soluble radioactivity from a labeled RNA we found no detectable RNase activity in the presence of 1 mM Mg^{2+} , Mn^{2+} , or Zn^{2+} under otherwise standard assay conditions. However, when a more sensitive gel assay was used to analyze the digestion patterns of radiolabeled RNAs we did detect significant cleavages in the presence of Mn^{2+} and Zn^{2+} , but only with large amounts of RNase X (data not shown). In particular, when Mn^{2+} replaced Ca^{2+} , ~100-fold more enzyme was needed for a similar level of RNase activity. When Zn^{2+} replaced Ca^{2+} , ~1000-fold more enzyme

Figure 3. SDS-PAGE analysis of Mono Q fractions. Aliquots (1.5 μl) of the fractions from the Mono Q column were electrophoresed in an 11% SDS-Laemmli gel and the gel was silver stained. Only a portion of the Mono Q column is shown (fractions 10 through 30). The peak of RNase X activity is indicated by the arrows and was found in fraction 15. The levels of Ca^{2+} dependent ribonuclease activity in fractions 12 through 17 were as follows: (12) 0.32 units/ μl , (13) 0.38 units/ μl , (14) 0.90 units/ μl , (15) 1.7 units/ μl , (16) 0.72 units/ μl , (17) 0.19 units/ μl . The chromatographic profile of a 36-kDa protein closely matched the level of Ca^{2+} dependent ribonuclease activity in the fractions. An essentially homogenous preparation of the 36-kDa protein was obtained by taking only the peak fraction 15. SM indicates an aliquot of the pooled fractions from the previous purification step, and M indicates size markers. A mixture of T7 RNA polymerase and *lac* repressor was electrophoresed in the lane between M and SM.

Figure 4. Activity of RNase X renatured in an SDS gel. Radioactively labeled RNA was incorporated into the separating portion of an 11% SDS-Laemmli gel during polymerization. Purified RNase X (0.6 or 1.5 μ g), acetylated BSA (2 μ g), and pre-stained molecular weight markers were run in the lanes indicated. Following electrophoresis the SDS was soaked out of the gel, the gel was cut in half, and one half was incubated in 1 mM Ca^{2+} while the other half was incubated without Ca^{2+} . Ribonuclease activity was then assayed by autoradiography of the gel. The relative positions and apparent molecular weights of the proteins used as size markers are indicated. After autoradiography the gel was stained with Coomassie Blue and the positions of the 36-kDa protein, RNase X, were seen to precisely match the observed Ca^{2+} dependent ribonuclease activity.

Figure 5. pH dependence of RNase X activity. The relative activity of RNase X at different pH values was measured by the release of acid soluble radioactivity from a synthetic radioactively labeled RNA. Assays were carried out for 3 min at 22°C with 0.02 units of RNase X in a 50 mM solution of the appropriate buffer. The buffers used were: sodium-acetate (pH 4.9), sodium-MES (pH 5.6), sodium-MES (pH 6.1), sodium-PIPES (pH 6.8), sodium-HEPES (pH 7.5), Tris-HCl (pH 8.2), Tris-HCl (pH 8.9), sodium-CHES (pH 9.6), and sodium-CAPS (pH 10.5). The reactions also contained 10 mM KCl, 0.5 mM CaCl_2 , 1 mM dithiothreitol, 100 μ g/ml acetylated BSA, and 100 ng of yeast ribosomal RNA.

was needed for a similar level of RNase activity. No cleavages were detected in the presence of Mg^{2+} even when 1000-fold more enzyme was added than was needed to obtain ribonuclease activity in the presence of Ca^{2+} .

The relative activity of RNase X at different pH values was measured and is shown in Fig. 5. The pH optimum is near 7.5 and ribonuclease activity drops significantly within two pH units on either side of this optimum. RNase X activity was found to be inhibited by moderate to high concentrations of KCl (> ~200 mM). We measured the activity of RNase X at decreasing

Figure 6. Digestion pattern of RNase X on a 91 nucleotide long 5' end-labeled RNA. RNase X digestions (0.03 units) were for the times indicated under standard assay conditions including 100 ng of carrier yeast ribosomal RNA per reaction. The control reaction corresponding to lane i was for 5 min with RNase X but contained no Ca^{2+} . The digestions were stopped by the addition of EGTA and the products were phenol extracted and analyzed on a denaturing polyacrylamide gel. Size markers produced by RNase T1 digestion are shown in lanes a-c.

concentrations of Ca^{2+} and found that maximal activity dropped sharply as the Ca^{2+} concentration decreased from 100 μ M to 10 μ M (data not shown). In these experiments the RNase activity was measured as the release of acid soluble radioactivity from a synthetic labeled RNA (see Materials and Methods) and no attempt was made to examine the possibility that at low Ca^{2+} concentrations more specific, and less extensive, cleavages were occurring.

We determined that RNase X has endonuclease activity by examining the cleavage products observed with RNA molecules radioactively labeled at either the 3' or the 5' end. In both cases radioactively labeled RNAs of intermediate length were produced during the early stages of digestion. This demonstrates that RNase X has endonuclease activity but does not rule out the possibility of additional exonuclease action. An example of this type of experiment is shown in Fig. 6 where an RNA labeled at the 5' end was briefly digested with a small amount of RNase X. Note that digestion of the RNA was Ca^{2+} dependent. Numerous cleavage sites were observed. Several of the strong cleavage sites were mapped to the same positions on this RNA when it was 3' end labeled. This is in agreement with the endonuclease mode of action. We have looked at the nucleotide sequences near the

cleavage sites for this RNA and for a handful of other small RNAs and we have not yet been able to identify a rule to predict the cleavage sites, although certain sequences were frequent targets. A more extensive analysis of the sequence and structure specificity of RNase X cleavage is currently underway.

DISCUSSION

It was surprising that only a 360-fold purification of a Ca^{2+} dependent ribonuclease from a *Xenopus* oocyte extract would yield a preparation containing a single 36-kDa protein. However, the data supporting the conclusion that the purified 36-kDa protein has Ca^{2+} dependent ribonuclease activity are convincing. First, the chromatographic pattern on Mono Q of the 36-kDa protein corresponds to the chromatographic pattern of Ca^{2+} dependent ribonuclease activity (Figs. 2 and 3). Second, Ca^{2+} dependent ribonuclease activity of the 36-kDa protein was observed after the protein was renatured in an SDS-Laemmli gel (Fig. 4). In this assay, protein samples were electrophoresed in an SDS-gel containing substrate RNA incorporated into the gel during polymerization. After removal of the SDS and renaturation of the protein, Ca^{2+} dependent RNase activity was observed at the position in the gel corresponding to the 36-kDa protein. We conclude that the purified 36-kDa protein, named RNase X, is a Ca^{2+} dependent ribonuclease.

Our purification of RNase X employed a procedure which may prove useful in the isolation of other Ca^{2+} sensitive proteins. We found that the binding of RNase X to heparin sepharose was Ca^{2+} dependent. Two runs over the same heparin sepharose column, first without Ca^{2+} and then with Ca^{2+} , under otherwise identical conditions, selected for a small group of Ca^{2+} sensitive proteins. Many of these proteins could then be obtained in nearly pure form (Figs. 2 and 3). This very effective purification step was essential to our purification procedure.

The purification data suggests that RNase X is present at relatively high concentrations in *Xenopus* oocytes. Taken at face value, a 360-fold purification to homogeneity implies a relative abundance of nearly 0.3% in the oocyte extract. Is the Ca^{2+} dependent ribonuclease activity of RNase X a new activity for a known protein? Probably not, because we have obtained the amino acid sequences of 3 tryptic peptides from the isolated 36-kDa protein and have not found any correspondence between the sequences of these peptides and the sequences currently available in the protein and nucleic acid sequence databases (data not shown). This suggests that the amino acid sequence of RNase X has not yet been identified and that this is the first report of the purification of this molecule.

RNase X requires Ca^{2+} for activity, and digests RNA but not DNA. Micrococcal nuclease isolated from *Staphylococcus aureus* is a thoroughly studied and widely used nuclease with an absolute requirement for Ca^{2+} (13). However, in contrast to RNase X, micrococcal nuclease digests both RNA and DNA substrates. There are other reports of Ca^{2+} stimulated ribonucleases, but in these cases the ribonucleases had reduced activity in the presence of Mg^{2+} and were not completely dependent on Ca^{2+} for activity (14, 15). In contrast, we have not observed any ribonuclease activity of RNase X in the presence of Mg^{2+} without Ca^{2+} . We suspect that calcium ions interact directly with RNase X for the following reasons. RNase X can be activated by the addition of 1 mM Ca^{2+} , then inactivated by the addition of 2 mM EGTA, and then reactivated by the addition of 3 mM Ca^{2+} (data not shown). The addition of Ca^{2+} also

slightly raises the temperature required to thermally inactivate RNase X activity (data not shown). At present we do not know if Ca^{2+} is directly involved in the catalytic mechanism of RNA hydrolysis or if Ca^{2+} is acting as an allosteric effector to bring the enzyme into an active conformation.

The Ca^{2+} dependence of RNase X activity coupled with the widespread use of Ca^{2+} as a second messenger for signal transduction raises some interesting possibilities for the biological function of RNase X. At the simplest level it is possible that changes in the intracellular level of Ca^{2+} directly modulate the activity of RNase X. Alternatively, it is possible that RNase X may have reduced, but potentially more sequence or structure specific, ribonuclease activity at very low Ca^{2+} levels or even in the absence of Ca^{2+} .

The early development of *Xenopus* embryos relies on the proteins and maternal mRNAs produced during oogenesis. Oocytes mature and are laid as eggs as a result of hormone stimulation. Fertilization of the eggs initiates a rapid series of early cleavage divisions during which time transcription does not occur (16, 17). Two processes are known to re-pattern the pool of translationally active maternal mRNAs. First, oocyte maturation triggers poly(A) addition and removal on different mRNAs coupled with differential translational recruitment of these messages (18). Second, a variety of particular maternal mRNAs are destabilized and degraded during this time. Some examples include the mRNAs for nucleoplasmin (19), bFGF (20), *c-myc* (21), *c-fos* (22), *c-mos* (23), and α -fodrin (24). The selective destruction of maternal mRNAs is likely to play an important role in the early developmental program, and it is possible that RNase X may be involved in this process. Particularly intriguing is the possibility that the well known increase in Ca^{2+} concentration which occurs at the time of fertilization of *Xenopus* eggs may directly activate RNase X (25–27).

The purification of a 36-kDa protein from *Xenopus* oocytes with Ca^{2+} dependent ribonuclease activity represents a first step toward understanding the structure, mechanism of action, and the biological function of this protein. The intracellular degradation of RNA molecules is of central importance to RNA metabolism and gene expression (2, 28). Purification of RNase X provides a starting point for the investigation of this enzyme and its involvement in regulating the levels of intracellular RNA.

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