The Cytoplasm of *Xenopus* Oocytes Contains a Factor That Protects Double-Stranded RNA from Adenosine-to-Inosine Modification

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Here we describe studies of double-stranded RNA (dsRNA) adenosine deaminase in *Xenopus laevis*, in particular during meiotic maturation, the period during which a stage VI oocyte matures to an egg. We show that dsRNA adenosine deaminase is in the nuclei of stage VI oocytes. Most importantly, we demonstrate that the cytoplasm of stage VI oocytes contains a factor that protects microinjected dsRNA from deamination when dsRNA adenosine deaminase is released from the nucleus during meiotic maturation. Our data suggest that the protection factor is a cytoplasmic dsRNA-binding protein or proteins that bind to dsRNA in a sequence-independent manner to occlude dsRNA from binding to dsRNA adenosine deaminase. The cytoplasmic double-stranded RNA-binding protein(s) does not bind to other nucleic acids and can be titrated at high concentrations of dsRNA. These studies raise the question of whether all dsRNA-binding proteins share endogenous substrates and also suggest potential means of regulating dsRNA adenosine deaminase in vivo.

The double-stranded RNA (dsRNA) adenosine deaminase (dsRAD), initially called the unwinding/modifying activity, was first detected in *Xenopus laevis* (4, 25) and subsequently in organisms throughout the animal kingdom (34; for reviews, see references 1, 2, and 17). This enzyme deaminates adenosines within dsRNA to produce inosines (24). Recently dsRAD has been purified from *Xenopus* eggs; this protein is a single subunit that migrates at 120 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (14). In vitro studies demonstrate that dsRAD can deaminate adenosines within intermolecular and intramolecular RNA duplexes (21, 29) but cannot act on adenosines within DNA or single-stranded RNA (ssRNA) (4, 33). Strong evidence indicating that dsRAD is responsible for RNA editing of mRNAs encoding the B subunit of a specific type of γ-glutamyl-activated ion channel has been presented (13). In addition to RNA editing, other biological roles for dsRAD have been proposed (for a review, see reference 2), and it is possible that dsRAD plays multiple roles in vivo.

Initial experiments in *X. laevis* showed that dsRAD could easily be detected by assaying the fate of dsRNA microinjected into the cytoplasm of eggs and early embryos (≤8 cells; 4, 25). However, the activity could not be observed in similar microinjections into the cytoplasm of oocytes or embryos at later stages of development, specifically, subsequent to the midblastula transition (post-MBT). Further experiments revealed that dsRAD was present in post-MBT embryos but was localized to the nucleus and thus not detected in early experiments involving cytoplasmic microinjections (5). dsRAD has also been shown to be nuclear in the somatic cells of mammals (34). Taken together, these results raised the possibility that dsRAD is in the nucleus of stage VI oocytes and exists in the cytoplasm of eggs and early embryos because it is released from the nucleus during meiotic maturation, the process by which a stage VI oocyte becomes an egg (for a review, see reference 31).

The studies reported here were designed to assay for the presence and intracellular location of dsRAD in *X. laevis* stage VI oocytes and during meiotic maturation. We observed that dsRAD was in oocytes, and as in post-MBT embryos, its activity was nuclear. Consistent with the breakdown of the nucleus during meiotic maturation, dsRNA was deaminated when microinjected into the cytoplasm of mature oocytes (eggs). However, we observed that dsRNA microinjected into the cytoplasm of stage VI oocytes prior to maturation was protected from modification when these oocytes were subsequently induced to mature. Experiments conducted to understand the nature of protection suggest that it is the result of a dsRNA-binding protein (dsRBP) present in the cytoplasm of *X. laevis* oocytes. The cytoplasmic factor binds to dsRNA of any sequence but not to other nucleic acids.

**MATERIALS AND METHODS**

Nucleic acid preparation. To prepare 800-bp dsRNA (800-dsRNA), sense and antisense transcripts of the chlorohemepicolyl acetyltransferase gene were synthesized in vitro with SP6 RNA polymerase, hybridized, and gel purified as previously described (4). Both strands were labeled during transcription with [α-32P]jATP or, when indicated, [γ-32P]UTP. The sense strand was gel purified as for 800-dsRNA and used as an ssRNA competitor (800-ssRNA). A SacI-BamHI fragment of pSp5CAT (12) encompassing the chlorohemepicolyl acetyltransferase gene was gel purified and used as a dsDNA competitor (800-dsDNA).

The 36-bp dsRNA molecule (36-dsRNA) was prepared by first synthesizing complementary single strands from a partially single-stranded DNA (ssDNA) template with T7 RNA polymerase as previously described (20). ssRNAs were collected by ethanol precipitation and subsequently gel purified after electrophoresis in 1× TBE (Tris-borate-EDTA) at 350 to 400 V on an 8 M urea–20% polyacrylamide gel followed by visualization with UV shadowing. Gel-pure single strands were hybridized by mixing approximately equal molar amounts of each strand in 10 mM Tris (pH 7.5)–0.5 mM EDTA, heating at 65°C for 10 min, and slowly cooling to room temperature. 36-dsRNA was...
gel purified as described for the single strands, except that electrophoresis was at 200 to 250 V. In the gel allowed greater resolution but did not result in denaturation of the duplex, since samples were not heated prior to electrophoresis and the gel was kept cool while running. 36-clDNA was 5' end labeled by treating the dsDNA with calf intestinal phosphatase, followed by T4 polynucleotide kinase and ['P]ATP, and then it was passed over a Chroma spin-10 column (Chontech) to remove unincorporated radiolabeled nucleotides.

The dsDNA and ssDNA competitors of approximately 100 bp or less in length were prepared from a plBluescript HS vector (Stratagene) containing a short insert (of no known significance) between the T7 and T3 promoters (provided by Janice Pata). A 133II fragment (139 bp) that included both phage promoters and intervening sequences was gel purified and used as a dsDNA competitor (100-dsDNA) and as a template for transcription of the ssDNA competitor (100-ssDNA). The 100-ssDNA competitor was transcribed by incubating 4 pmol of the BstEII dsDNA fragment with 50 µg of ['H]UTP and 2 U of T3 RNA polymerase per µl according to the manufacturer's specifications (Stratagene).

Preparation of oocytes. Female frogs were injected subcutaneously with 250 µl of 0.2 U of pregnant mare serum gonadotropin per µl 1 day before the oocytes were surgically removed. Individual oocytes were removed from their follicles according to the methods by Cankan's counting of trial injections. For nuclear microinjections, the oocytes were centrifuged in a Beckman tabletop GP centrifuge at 1,600 rpm for 10 min to force the nucleus to the surface. The position of the nucleus could be seen at the center of the animal hemisphere after centrifugation. 800-dsDNA was microinjected into the area observed to be the nucleus. Five to ten whole oocytes were collected at each time point. homogenized in 200 µl of 5% protease K buffer (5% protease K buffer is 250 mM Tris pH 7.5, 0.5 mM EDTA, 0.1% sodium dodecyl sulfate, 1 mg of proteinase K per ml), and incubated at 37°C for 30 min. Eighteen percent of the adenoviruses were observed to be deaminated (see Fig. 1B) whereas 50% deamination had been observed in other studies (5). Immediately following a nuclear microinjection, some 800-dsDNA was detected in the cytoplasm. Furthermore, when nuclei that had been microinjected with 800-dsDNA were dissected from the oocytes and analyzed without the contents of the cytoplasm, 50% of the adenoviruses were observed to be deaminated (27). Therefore, we attributed the low level of deamination to leakage of 800-dsRNA out of the nucleus before deamination occurred. The homogenized oocytes were phenol and chloroform extracted, and the RNA was collected by ethanol precipitation. The RNA was either electrophoresed on a native 4% polyacrylamide gel (4) or analyzed by thin-layer chromatography (TLC).

TLC analysis and inosine quantitation. For TLC analysis, dsDNA was digested to mononucleotides with P1 nuclease as follows. The precipitated RNA was resuspended in 40 µl of 25 mM Tris (pH 7.5)-0.1 mM ZnCl2, heated at 90°C for 2 min, and immediately placed on ice. P1 nuclease (1 U) (Boehringer Mannheim) was added, and the mixture was incubated at 70°C for 30 min. To ensure complete digestion, the samples were again heated to 90°C, cooled on ice, and incubated for 20 min at 70°C with an additional unit of P1 nuclease. Prior to analysis, samples were phenol, chloroform, and ether extracted, dried in a Speed Vac concentrator, and resuspended in 5 µl of water. The resulting mononucleotides were chromatographed in one dimension on a polyethyleneimine (PEI)-cellulose TLC plate with 3 M sodium acetate (pH 3.5) as the solvent. When, or, when indicated, on a celite TLC plate with saturated (NH4)2SO4, 0.1 M sodium acetate (pH 6.0)-isopropanol (79:19:2, by volume) as the solvent. The 3'-P-labeled 5'-a and 5'-o markers were prepared as previously described (5). Each radioactive spot was quantitated with a Molecular Dynamics Phosphorlmager. The percent inosine was calculated by dividing the radioactivity in the inosine spot by the total radioactivity (Pa plus Pa origin). Duplicate experiments that resulted in different amounts of undegraded material contained the same percentage of inosine, indicating that the size of the origin spot did not alter the accuracy of quantitation.

Preparation of nuclear and cytoplasmic extracts. Nuclei were dissected by hand as previously described (9) with a few modifications. A micropipette tip was pulled into a needle and micro-dissecting forceps with serrated ends were used to dissect the nuclei out. Furthermore, to maintain the activity of the extracts, the oocytes were dissected in 0.5°-TGKED buffer (0.5°-TGKED buffer is 25 mM Tris[pH 7.9], 12.5% glycerol, 25 mM KCl, 0.25 mM diethiothreitol, and 0.05 mM EDTA) rather than buffer X, and the oocytes were stored in MBs and transferred to 0.5°-TGKED buffer immediately before dissection. The nucleated cells were diluted with an equal volume of 0.5°-TGKED buffer and microcentrifuged in an Eppendorf 5415C centrifuge at maximal speed for 30 min at 4°C. The supernatant between the lipid pellicle layer and the yolk platelet pellet was saved as the cytoplasmic extract. The nuclei were diluted to the same final volume as the cytoplasmic extract and vortexed to lyse the nuclei. Both nuclear and cytoplasmic extracts were stored on ice at 4°C.

In vitro assays. Unless indicated, in vitro assays consisted of a 15-min preincubation, followed by a 15-min incubation, both at 25°C. The preincubation reaction mixture contained 1 mM ATP, 15 mM EDTA, 1,600 U of RNasin per ml, and 12.5 mM Tris (pH 8.0). As indicated, the figures or legend), the following were included in the preincubation mixture in a final volume of 10 to 12 µl: 2.5-4.5 oocyte equivalents (eq) of cytoplasmic extract (5 µl), 1.5 eq of nuclear extract (3 µl), 800-dsRNA (1 fmol in 1 µl) when, or, indicated, 0.1, 0.2, or 0.5 fmol in 1 µl), or competitors (10 fmol of 100-sRNA, 800-sRNA, 100- dsDNA, or 808-dsDNA in 1 µl). were added prior to the dsDNA. Extracts were prepared in 0.5°-TGKED buffer and the nucleic acids in water; thus, when necessary, reaction volumes were maintained with these ingredients. The preincubation reactions were stopped by stopping 5 µl of 5% proteinase K buffer and incubating at 37°C for 30 min. The samples were then phenol and chloroform extracted, and the RNA was precipitated with ethanol. For reaction mixtures that included 800-dsDNA, the RNA products were analyzed on a 4% polyacrylamide gel in 1 X TBE at 200 to 250 V or by TLC as
described above. Reactions with the 36-dsRNA were performed as described above, except that the cytoplasmic and nuclear extracts, stop buffer volumes, and reaction times were doubled and analysis was done with an 8 M urea-20% polyacrylamide gel in 1X TBE at 200 to 250 V.

Cytoplasmic extract was inactivated by heating 2.5 eq of cytoplasmic extract at 65°C for 10 min followed by cooling on ice, prior to being added to the reaction mixture (see Fig. 4, bar 3). Cytoplasmic nucleic acids were isolated from 2.5 eq of cytoplasmic extract by phenol and chloroform extraction followed by ethanol precipitation. The nucleic acids were resuspended in 5μl of water and substituted for the cytoplasmic extract in the reaction mixture (see Fig. 4, bar 4).

RNA helicase assays with RNase A. In vitro RNA helicase experiments were performed by incubating 1 fmol of 800-dsRNA and 5 fmol of 800-dsRNA in reaction buffer containing 1 mM ATP, 15 mM EDTA, 4 U of RNasin per μl, 12.5 mM Tris (pH 8.0), and either 2.5 eq of cytoplasmic extract (5 μl) or 5 μl of 0.5× TGKED buffer for 30 min at 25°C. One microliter of RNase A (0.001 U/μl) or 1 μl of 0.5× TGKED buffer was then added to the reaction mixture, and the incubation was continued for 15 min at 25°C. The reactions were stopped and analyzed as described below for reactions with partially pure dsRAD.

In vitro modification with a partially pure preparation of dsRAD. A partially purified extract of dsRAD was prepared as previously described (24). Five micrograms of extract was added for every femtomole of 800-dsRNA and reacted in a mixture of 1 mM ATP, 12.5 mM Tris (pH 8.0), 15 mM EDTA, and 1,600 U of RNasin per ml for 1 h at 25°C. Reactions were stopped by adding 5 μl of 5× proteinase K buffer and incubating at 37°C for 30 min. After phenol and chloroform extractions, the RNA was collected by ethanol precipitation and analyzed on a 4% polyacrylamide gel in 1X TBE at 200 to 250 V.

RESULTS

dsRAD is nuclear in stage VI oocytes. Microinjection experiments were performed to assay for the presence of dsRAD in stage VI oocytes. An ~800-bp dsRNA, internally labeled with [α-32P]ATP (800-dsRNA) was microinjected into oocyte nuclei, and the RNA products were extracted and analyzed by electrophoresis (Fig. 1A) or TLC (Fig. 1B). 800-dsRNA microinjected into nuclei exhibited an altered electrophoretic mobility (Fig. 1A). As previously established (5), an altered gel mobility is indicative of modification, since the structure of dsRNA changes when an AU base pair is converted to the less stable IU base pair. TLC analysis of the 5′ mononucleotides derived from the injected 800-dsRNA showed that the production of inosine correlated with the slower electrophoretic mobility (Fig. 1B). In this experiment, 18% of the adenosines in 800-dsRNA were converted to inosines within 30 min after injection (mean ± standard deviation of eight independent microinjections = 16.8± 3.5%; also see Materials and Methods). In contrast, when 800-dsRNA was injected into the cytoplasm, inosine was not detected for up to 9 h after injection (Fig. 1C). We concluded that dsRAD was in the nucleus but not the cytoplasm of stage VI oocytes.

dsRNA microinjected into the cytoplasm of stage VI oocytes is not modified when dsRAD is released into the cytoplasm during meiotic maturation. Stage VI oocytes are arrested in G2 of meiosis 1 (for a review, see reference 31). When stimulated with progesterone, these oocytes resume meiosis and go through the process of meiotic maturation. During meiotic maturation, the nuclear membrane breaks down and the contents of the nucleus are released into the cytoplasm, an event known as germinal vesicle breakdown (GVBD). Consistent with the expected release of dsRAD into the cytoplasm during maturation, we observed that 800-dsRNA injected into the cytoplasm after GVBD was modified (Fig. 2, lanes B). In
FIG. 2. 800-dsRNA injected into the cytoplasm of oocytes is protected from dsRAD released during meiotic maturation. Lanes A. PE1-cellulose TLC analysis of 800-dsRNA that was injected into the cytoplasm of stage VI oocytes prior to maturation and extracted after the oocytes had undergone GVBD. Oocytes were collected 2, 4, and 6 h after GVBD. Lanes B. PE1-cellulose TLC analysis of 800-dsRNA that was injected into maturing oocytes immediately after GVBD and collected 2, 4, and 6 h after injection. Mononucleotides marks are as described in the legend to Fig. 1.

this experiment, 8% of the adenosines were modified after 2 h (mean ± standard deviation of four independent microinjections = 7.2% ± 0.5%). However, when 800-dsRNA was injected into the cytoplasm of stage VI oocytes and then maturation was induced, inosine was essentially undetectable even 6 h after GVBD (Fig. 2, lanes A). This result was quite surprising. Our experiments indicated that dsRAD was active when it was released into the cytoplasm at GVBD (Fig. 2, lanes B); hence, cytoplasmic dsRNAs should be exposed to dsRAD and modified.

It seemed possible that injecting dsRNA into the cytoplasm prior to maturation inactivated dsRAD. This possibility was tested with a double-label experiment as follows. [3H]800-dsRNA was injected into the cytoplasm of stage VI oocytes. The oocytes were then induced to mature, and at GVBD, [32P]800-dsRNA was injected. A TLC analysis of the extracted RNA (Fig. 3A) clearly showed the presence of [32P]IMP, ruling out the possibility that dsRAD was inactivated by the first injection.

We also considered the possibility that dsRNA injected into the cytoplasm prior to GVBD was covalently altered in a way that precluded its modification. To test this possibility, 800-dsRNA was injected into the cytoplasm of stage VI oocytes. These oocytes were induced to mature, and 4 h after GVBD, the RNA was extracted. The deproteinized 800-dsRNA was then incubated with a partially purified extract of dsRAD and analyzed by electrophoresis. The 800-dsRNA migrated more slowly, indicating it was modified (Fig. 3B, lane 2). We concluded that dsRNA injected into the cytoplasm of stage VI oocytes was a competent substrate for dsRAD released from the nucleus at GVBD.

The cytoplasm of stage VI oocytes contains a factor that protects dsRNA from deamination. The simplest explanation for the lack of deamination of 800-dsRNA injected prior to GVBD was that the oocyte cytoplasm contained a factor that bound and protected dsRNA from modification when dsRAD was released from the nucleus. One alternative explanation, that a factor in the cytoplasm was acting directly to inhibit dsRAD, seemed unlikely, since dsRNA injected after GVBD was deaminated (Fig. 2, lanes B). To explore the mechanism of protection of dsRNA from modification, an in vitro assay was developed. Cytoplasmic and nuclear extracts were prepared by manually dissecting stage VI oocytes. The in vitro microinjection results could be reproduced using the in vitro extracts. dsRNA incubated with 1.5 q of nuclear extract was modified (Fig. 4, bar 1), while modification was diminished when the dsRNA was incubated with 2.5 q of cytoplasmic extract prior to addition of the nuclear extract (Fig. 4, bar 2). The reduction of modification afforded by the cytoplasmic extract was sensitive to heat, suggesting that protection was not due to a small molecule or ion (Fig. 4, bar 3). Since high concentrations of dsRNA inhibited dsRAD (14), it was important to determine if protection was due to the presence of dsRNA in the oocyte cytoplasm. Nucleic acids were extracted from the cytoplasmic fraction and added to the in vitro reaction mixture (Fig. 4, bar 4). The nucleic acids did not inhibit dsRAD. The above data suggested that protection of dsRNA from deamination was due to the presence of a cytoplasmic dsRNA-binding protein or proteins (cyto-dsRBP).

It seemed possible that cyto-dsRBP was an RNA helicase. An RNA helicase could unwind the dsRNA and thus eliminate it as a substrate for dsRAD. To investigate this possibility, we
incubated 800-dsRNA and 800-ssRNA with 2.5 eq of cytoplasmic extract for 30 min and then added 0.001 U of RNase A. The 800-ssRNA was degraded, while the 800-dsRNA remained stable (Fig. 5, lane 6), indicating that dsRNA incubated in the cytoplasmic extract remains in duplex form and that cyto-dsRBP is not an RNA helicase.

Although the amount of deamination by the nuclear extract was diminished by preincubating 800-dsRNA with the cytoplasm, protection of the 800-dsRNA in vitro was not complete and some inosine was produced (Fig. 4, bar 2). This was in contrast to observations made in vivo, where deamination of 800-dsRNA injected into the oocyte cytoplasm prior to GVBD was nonexistent following maturation. One difference between the in vivo and in vitro conditions was the length of time the 800-dsRNA was exposed to the cytoplasm prior to exposure to dsRAD. In our in vivo experiments, maturation occurred 2 to 3 h after progesterone addition; hence, 800-dsRNA injected at the time of progesterone treatment was incubated with the cytoplasm alone longer than the in vitro preincubation time of only 15 min. To determine if the length of time dsRNA was exposed to the cytoplasmic extract was important for protection, 800-dsRNA was incubated with the cytoplasmic extract for increasing times prior to adding the nuclear dsRAD fraction. Longer preincubation times decreased the percent inosine to levels similar to those observed in vivo (Fig. 6, closed circles), demonstrating that a longer preincubation time was required for complete protection in vitro. The cytoplasmic and nuclear extracts remained active throughout the preincubation time (Fig. 6, open triangles and open circles); moreover, preincubating the cytoplasmic and nuclear fractions together prior to the addition of 800-dsRNA did not increase protection.

![FIG. 5. The cytoplasmic extract does not contain an RNA helicase. An autoradiogram of a 4% polyacrylamide gel with lanes loaded as follows: 1, untreated 800-ssRNA; 2, untreated 800-dsRNA; 3, 800-dsRNA and 800-ssRNA incubated in buffer; 4, 800-dsRNA and 800-ssRNA incubated in cytoplasmic extract; 5, 800-dsRNA and 800-ssRNA incubated in buffer followed by the addition of RNase A; 6, 800-dsRNA and 800-ssRNA incubated in cytoplasmic extract followed by the addition of RNase A. Some degradation of the 800-ssRNA occurred in the cytoplasmic fraction prior to the RNase A addition, suggesting the presence of an endogenous single-stranded RNase in the cytoplasmic fraction (lane 4).](image)

![FIG. 6. Preincubating dsRNA for longer times with the cytoplasmic extract decreases the extent of deamination by dsRAD. Preincubation time is plotted against percent inosine. Following the various preincubation times, all samples were incubated with dsRAD for a constant time of 15 min. The datum points represent the in vitro reactions as follows. Symbols: open circles, nuclear extract preincubated alone and then incubated with dsRNA; closed circles, dsRNA preincubated with cytoplasmic extract and then incubated with nuclear extract; closed triangles, cytoplasmic and nuclear extracts preincubated together and then incubated with dsRNA; open triangles, cytoplasmic extract preincubated alone and then incubated with nuclear extract and dsRNA, added quickly in sequence. Analysis was by TLC on cellulose plates. Each time point was performed a minimum of three times.](image)
observation suggested that both dsRAD and cyto-dsRBP are present after maturation and compete for binding to a dsRNA substrate injected at this time.

cyto-dsRBP binds specifically to dsRNA. cyto-dsRBP, like dsRAD (4, 21), was not sequence specific and also could act on intermolecular and intramolecular dsRNA. Modification of a different dsRNA molecule, a synthetic 36- dsRNA, was completely repressed by the addition of the cytoplastic fraction (Fig. 7). The electrophoretic mobility of modified 36-dsRNA is different from that of the 800-dsRNA, and 36-dsRNA resolves into discrete slower-mobility species (Fig. 7, lane 2 (23)). None of the slower-mobility species were produced when the 36-dsRNA was preincubated with the cytoplastic fraction prior to the addition of the nuclear fraction (Fig. 7, lane 3). A 31-bp intramolecular stem-loop RNA was also protected by cyto-dsRBP (26).

However, cyto-dsRBP was not a general, sequence-independent nucleic acid-binding protein, as demonstrated by competition studies. When a 100-fold molar excess of 100-dsDNA or 800-dsDNA was added to the in vitro reaction mixtures, neither modification by the nuclear extract nor protection by the cytoplastic extract was diminished (Fig. 8, compare - lanes to dsDNA lanes). The same result was observed when a 1,500-fold molar excess of the dsDNA competitors was added to the reaction mixtures (150 fmol of 100-dsDNA or 800-dsDNA versus 0.1 fmol of 800-dsRNA substrate) (26). Similarly, when a 100-fold molar excess of a 100-sRNA or 800-sRNA was added to the in vitro reaction mixtures, modification and protection were equivalent to the no-competitor lanes (Fig. 8, compare - lanes to sRNA lanes). Even though the 100- and 800-sRNAs may contain some intramolecular secondary structure, they did not compete for binding to cyto-dsRBP. We have not determined the number of contiguous base pairs required for binding to cyto-dsRBP and surmise that the putative intramolecular structures formed by the sRNAs are not long enough to compete for binding or that tertiary interactions preclude recognition of intramolecular duplexes.

In ruling out the possibility that a dsRNA helicase was responsible for diminished modification, we observed low levels of a cytoplasmic single-stranded RNAse that resulted in degradation of approximately half of the sRNA (Fig. 5, lane 4). Thus, it was possible that the existing molar excess of sRNA competitor was essentially only 40- to 50-fold. To confirm that the sRNA competitor was not competing for cyto-dsRBP, a 1,500-fold molar excess of either 100-sRNA or 800-sRNA (150 fmol of 100-sRNA or 800-sRNA versus 0.1 fmol of 800-dsRNA substrate) was added to the assay mixture.

Protection was still observed when a 1,500-fold excess of 100- or 800-sRNA was added to the reaction mixtures (26). In addition, microinjection of dsRNA or sRNA competitors with a dsRNA substrate did not result in decreased protection during maturation, supporting the in vitro data (26).

In contrast, when the 800-dsRNA concentration was increased by 22- or 43-fold in the in vitro reaction mixtures, protection was no longer observed (Fig. 9). The total inosine amount was almost equivalent in the nuclear-only lanes (N) at each of the 800-dsRNA concentrations, even though the percent inosine decreased, indicating that dsRAD was saturated under these conditions. Nevertheless, at higher concentrations of 800-dsRNA, the percent inosine and the total amount of inosine were equivalent with (NC) or without (N) cytoplastic extract, revealing that cyto-dsRBP could be titrated with excess dsRNA. Once more, similar experiments performed in vivo confirmed these in vitro observations. Specifically, dsRNA was not protected from degradation during maturation if a 10- or 20-fold increase of 800-dsRNA was injected as described in the legend to Fig. 2, lanes A (26).

**DISCUSSION**

Here we show that dsRAD is in the nuclei of stage VI oocytes and is released into the cytoplasm during meiotic maturation. Surprisingly, dsRNA microinjected into the cytoplasm of a stage VI oocyte is not modified when dsRAD is released from the nuclei during maturation. Our data indicate that a protein (or proteins) present in the oocyte cytoplasm binds to dsRNA, occluding it from binding to dsRAD and thus preventing degradation. The cyto-dsRBP appears to be specific for dsRNA since it cannot be titrated with dsDNA or ssRNA but can be titrated with dsRNA. cyto-dsRBP recognizes intermolecular as well as intramolecular dsRNA structures in a sequence-independent manner.

The nucleic acid-binding properties of cyto-dsRBP place it in the group of proteins collectively known as dsRBPs (for examples, see references 3, 8, 11, 15, and 32). dsRBPs,
including dsRAD and cyto-dsRBP, all bind dsRNA regardless of sequence but will not bind other nucleic acid structures. Phenomena such as we have described here have precedence among known dsRBPs, in particular those known to play a role in the mammalian antiviral response (for a review, see reference 28). dsRNA elicits a cellular antiviral response by binding to and activating the dsRBP called dsRNA-dependent protein kinase (PKR). Upon binding dsRNA, PKR is autophosphorylated and in turn phosphorylates p-eIF-2-a, resulting in the inhibition of protein synthesis. Reoviruses and vaccinia virus prevent the activation of PKR by expressing the dsRBPs sigma 3 and p25, respectively, which serve to occlude dsRNA from binding to PKR (6, 16, 19). The abilities of the reovirus protein sigma 3 and the vaccinia virus protein p25 to bind dsRNA and sequester it from PKR are similar to cyto-dsRBP's ability to mask or occlude dsRNA from dsRAD. The studies reported here, as well as the work cited in regard to viral dsRBPs, raise the possibility that dsRBPs, since they are not sequence specific, carry out their specific biological functions through a complex interplay with other dsRBPs, all competing for the same endogenous substrates.

As mentioned, dsRAD has recently been implicated in RNA editing of certain glutamate receptor RNAs (13). Editing of glutamate receptor RNA requires a dsRNA structure formed between exonic and intronic sequences and therefore must occur in the nucleus prior to splicing. At present, it seems unlikely that cyto-dsRBP is involved in the regulation of glutamate receptor RNA editing, since cyto-dsRBP is located in the cytoplasm. However, it is possible that other dsRBPs exist in the nucleus and somehow provide a means of regulating editing by dsRAD.

The existence of cyto-dsRBP suggests that dsRAD functions in the nucleus in Xenopus oocytes, with cyto-dsRBP serving to prevent promiscuous modification of cytoplasmic dsRNA during GVBD. Interestingly, in the silkworm moth Bombyx mori a protein factor that protects or inhibits deamination of dsRNA by dsRAD is present (30). As in Xenopus oocytes, the opposing activities are found in different cellular compartments, cytoplasm versus nucleus. However, in this case it is unclear whether the protein factor acts on dsRAD or directly interacts with dsRNA to prevent modification. However, it is interesting that another system may regulate dsRNA deamination.

We can envision a situation where cyto-dsRBP fulfills diverse roles in vivo. For example, cyto-dsRBP may be involved in regulating translation and thus explain the translational inefficiency of hairpin-containing mRNAs in Xenopus oocytes (10). In this regard, cyto-dsRBP is significant to all studies involving microinjection of structured RNAs into Xenopus oocytes: observations made during microinjection of structured RNAs may be influenced by this factor.

Several years ago, Kimmelman and Kirschner (18) reported that in Xenopus oocytes, the sense and antisense transcripts of basic fibroblast growth factor (bFGF) hybridize to form dsRNA molecules. However, in this case it is unclear whether the protein factor acts on dsRAD or directly interacts with dsRNA to prevent modification. However, it is interesting that another system may regulate dsRNA deamination.

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