Proc. Natl. Acad. Sci. USA
Vol. 74, No. 9, pp. 3835–3839, September 1977
Cell Biology

Presence of tadpole and adult globin RNA sequences in oocytes of *Xenopus laevis*

(oogenesis/embryogenesis/complementary DNA/molecular hybridization)

S. M. PERLMAN, P. J. FORD*, AND M. M. ROSBASH

Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02154

Communicated by Joseph G. Gall, May 13, 1977

ABSTRACT **Complementary DNA transcribed from adult *Xenopus laevis* globin mRNA was used to assay ovary RNA from *Xenopus* for the presence of globin sequences by RNA·cDNA hybridization. These sequences are present at approximately the same concentration as the majority of poly(A)-containing ovary sequences. The sequences are also found at approximately 200,000 copies per cell in poly(A)-containing RNA extracted from mature oocytes.**

To rule out contamination of the oocytes with somatic cells, two additional experiments were performed. First, RNA isolated from ovulated unfertilized eggs, which are devoid of somatic cells, was also shown to contain the globin sequences. Second, globin mRNA was isolated from *Xenopus* tadpoles. Adult globin mRNA is free of the tadpole sequence and no homology was detected between adult and tadpole globin RNA. The ovary was shown to contain tadpole globin RNA at nearly the same concentration as the adult sequences. Thus, the results cannot be explained by contamination with erythroid cells which should contain only the adult sequence.

The swimming tadpole, which possesses an active circulatory system, was also assayed for the tadpole and adult globin sequences. Whereas the adult sequences are present at approximately the same concentration as in the mature oocyte, the concentration of the tadpole sequences increases at least 300-fold in the first 3 days following fertilization.

The lampbrush chromosomes of amphibian oocytes are the sites of intense transcriptional activity (1). Although the RNA sequences present in oocytes have been partially characterized, many questions remain to be answered (2–4). One important question is the extent to which the oocyte sequences are specific for oogenesis and early development.

One approach to this problem is to assay ovary RNA for mRNA sequences that code for proteins normally associated with terminally differentiated cells. In this report, a successful search for the globin mRNA sequence of *Xenopus laevis* in the oocyte is described. In addition, the number of copies of this sequence per oocyte was found to be not markedly different from the number of copies of the majority of other poly(A)-containing sequences per oocyte.

MATERIALS AND METHODS

Preparation of RNA. *Ovary RNA.* Ovaries were removed from mature *X. laevis* females, washed with Barth X medium (5), and resuspended in Barth X medium containing 1% Pronase and 25 mM EDTA. After 10–15 min, the individual oocytes were essentially free of follicle cells. In some experiments, the oocytes were separated according to size as described (2). The oocytes were washed and the RNA was extracted by the method of Kirby as described (2, 6).

Egg RNA. Unfertilized ovulated eggs were dejellied with Barth X containing 2% cysteine (7). The eggs were washed several times with Barth X and the RNA was extracted by the method of Kirby (2, 6) as for ovary RNA.

Tadpole RNA. Tadpoles were harvested at stage 41, several hours before feeding normally begins (8). A cytoplasmic extract was prepared (S. M. Perlman and M. M. Rosbash, unpublished data), and RNA was extracted by the method of Kirby (2, 6) as above.

Adult globin RNA. Five females were made anemic by injection into the dorsal lymph sac of 5 mg of acetylphenylhydrazine on 2 consecutive days (9). Blood was collected by heart puncture 3 weeks after the first injection. The blood was washed with Barth X and the cells were lysed with buffer B (0.1 M NaCl 0.02 M Tris·HCl, pH 7.6/0.01 M $MgCl_2$) containing 0.5% Triton X-100. Nuclei were pelleted for 2 min at 2000 rpm; sodium dodecyl sulfate at a final concentration of 0.5% and EDTA at 0.02 M were added to the resultant cytoplasm. RNA was extracted with phenol and chloroform as described (10).

Tadpole globin RNA. Tadpoles were grown for 6 weeks in the presence of thiourea (400 μg/ml). [Thiourea halts development at approximately stage 53 of development, before metamorphosis has begun (8, 11).] Fifty tadpoles were injected in the hind limb bud with 25 μl of acetylphenylhydrazine (0.6 mg/ml) either once or on 2 successive days. Two weeks later, blood was extracted by heart puncture and washed with Barth X. RNA was extracted as for adult globin RNA.

Preparation of Poly(A)-containing RNA and Complementary DNA. Poly(A)-containing RNA was isolated from total RNA by passage over oligo(dT)-cellulose (T-2) (Collaborative Research) as described (12).

^{32}P- and ^{3}H-labeled complementary DNA (cDNA) were synthesized and purified as described except that [^{32}P]dCTP was used at a final concentration of 32 μM (13).

RNA·DNA Hybridization and Assay. RNA·DNA hybridization was performed as described (14) except that the reactions were at 70° in 1.0 M NaCl/5 mM EDTA/10 mM Tris·HCl, pH 7.4. $C_{ro}t$ (product of RNA concentration and time in mol·sec/liter) values were determined in one of two ways. For total RNA, $C_{ro}t$ was determined from the optical density by assuming 1 mg = 25 A_{260} units. For poly(A)-containing RNA, the amount of poly(A) was determined by hybridization with radioactive poly(U) (15). Poly(A)-containing RNA from the tadpole, oocyte, and egg was assumed to have a length of 1500 nucleotides and a 5% poly(A) content. The $C_{ro}t$ values were corrected to 0.18 M Na^+ at 60° ($C_{ro}t_e$) as described (13, 16). Hybrid formation was monitored with nuclease S1 as described (13).

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Abbreviations: cDNA, complementary DNA; $C_{ro}t$, product of RNA concentration and time in mol·sec/liter; $C_{ro}t_e$, $C_{ro}t$ corrected to 0.18 M Na^+ at 60°.

* Permanent address: Department of Molecular Biology, University of Edinburgh, Edinburgh, Scotland.

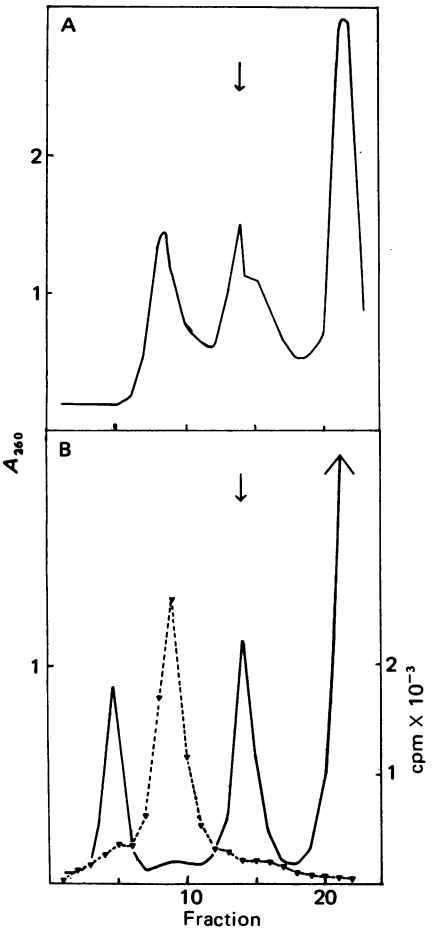

FIG. 1. Sedimentation analysis of erythrocyte RNA. Erythrocyte RNA from anemic adult and tadpole *Xenopus laevis*, extracted as described in *Materials and Methods*, was analyzed by centrifugation for 22 hr at 24,000 rpm in the Beckman SW-40 rotor in 15–30%/sucrose 0.1 M NaCl/0.01 M Tris·HCl, pH 7.4/0.001 M EDTA/0.5% sodium dodecyl sulfate. Sedimentation is from left to right. The arrows indicate the position of 18S rRNA. (*A*) Adult blood. The optical density pattern is shown. (*B*) Tadpole blood. The size of the poly(A)-containing RNA was determined by hybridization with [^{3}H]poly(U) as described (15) (dotted line). Also shown is the optical density pattern (solid line).

RESULTS

Characterization of cDNA Probes. In order to assay for the globin RNA sequence in oocyte RNA, it is necessary to obtain and characterize a relatively pure probe. Consequently, 10S RNA was isolated from the blood of anemic toads and tadpoles as described in *Materials and Methods*. The final steps of the purification are shown in Fig. 1. Adult 10S RNA was isolated by one cycle of sucrose gradient velocity sedimentation and one passage over oligo(dT)-cellulose (Fig. 1*A*). Because only small amounts of blood were obtained from tadpoles, less rigorous isolation methods were employed in this case (Fig. 1*B*). Total cytoplasmic RNA from anemic tadpole erythrocytes was centrifuged through a sucrose gradient and fractions were assayed by hybridization with radioactive poly(U) as described (15). A prominent peak of poly(A)-containing RNA sediments at 10 S. This region of the sucrose gradient was collected and used without further fractionation. Both 10S RNAs were transcribed into cDNA with reverse transcriptase as described in *Materials and Methods*. The resultant DNAs were 450–550 nucleotides in length (data not shown).

To demonstrate that these RNA preparations represented a limited number of 10S RNA sequences, the two cDNA preparations were hybridized to their template RNAs in RNA excess. As previously described, the rate of such a reaction is a funtion of the sequence complexity of the driver RNAs (17, 18). As shown in Fig. 2, the $C_{ro}t_{1/2}$ was 2–3 mmol·sec/liter. This value is similar to that obtained with rabbit globin mRNA and suggests that each RNA population consists of two to four 10S RNA species (19). The two radioactive cDNA probes were also hybridized to the heterologous 10S RNAs. As shown in Fig. 2*A*, there was little, if any, hybridization between tadpole cDNA and adult 10S RNA, indicating a lack of sequence homology between the adult and tadpole globin sequences as assayed under these conditions. The lack of reactivity also indicates the virtual absence of tadpole sequences in the adult 10S RNA preparation.

In contrast, the data in Fig. 2*B* show that the tadpole globin RNA did hybridize with adult globin cDNA although at a $C_{ro}t_{1/2}$ that was 30-fold greater than for the homologous hybridization. The difference in reaction rate suggests that the tadpole globin RNA preparation contained approximately 3% adult globin RNA. These data indicate that both cDNA preparations are sufficiently pure for testing for the presence of both adult and tadpole globin sequences in ovary RNA.

Assay of Ovary RNA for Adult and Tadpole Globin Sequences. Total *Xenopus* ovary RNA was assayed for the presence of adult and tadpole globin sequences. The hybridizations were performed with globin [^{32}P]cDNA and *Xenopus* ovary [^{3}H]cDNA (cDNA transcribed from poly(A)-containing RNA isolated from *Xenopus* ovary). The rate and extent of the hybridization of the ovary [^{3}H]cDNA provides an internal standard to which the hybridization of globin cDNA can be compared. The rate of the hybridization of ovary cDNA with ovary RNA has been carefully analyzed and can be adequately explained by the presence of two abundance classes of ovary RNA sequences; the most abundant, least complex class contains approximately 1000 different sequences and the least abundant,

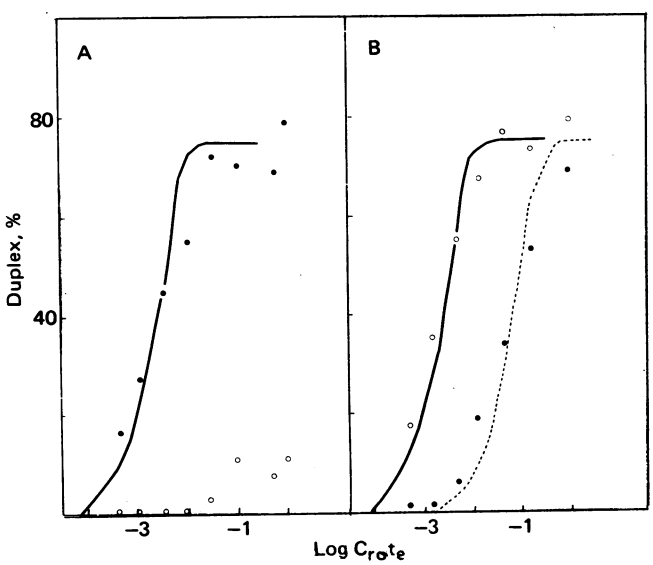

FIG. 2. Hybridization of adult and tadpole globin cDNAs with homologous and heterologous templates. Adult and tadpole cDNA, synthesized as described in *Materials and Methods*, was hybridized with adult (*A*) and tadpole (*B*) 10S RNA. To calculate $C_{ro}t$ values, globin RNA was assumed to contain 10% poly(A) measured by hybridization to [^{3}H]poly(U) (17). ●, Adult [^{32}P]cDNA; ○, tadpole [^{3}H]cDNA. The solid and dotted lines are ideal first-order curves of the form $D/C_0 = 1 - e^{-kC_{ro}t_e}$ in which D/C_0 is the fraction of the cDNA double stranded at time t_e, k is the rate constant, and $C_{ro}t_e$ is the equivalent $C_{ro}t$ value. Maximum value of D/C_0 was 75%.

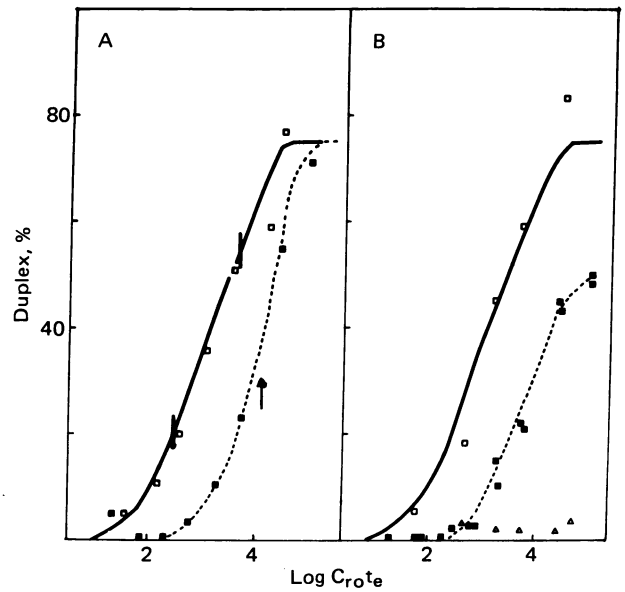

FIG. 3. Assay of ovary RNA for globin sequences. Ovary RNA was hybridized with ovary [^{3}H]cDNA (cDNA transcribed from poly(A)-containing ovary RNA) and globin [^{32}P]cDNA. The ratio of ovary RNA to globin cDNA was 2 to 4 × 10^7. The hybridization of ovary poly(A)-containing RNA to ovary cDNA has been characterized (S. M. Perlman and M. M. Rosbash, unpublished data). The solid line is a two-component curve fitted to the data determined in several experiments. The data shown have not been separately fitted to a curve. (*A*) Adult globin cDNA. The vertical bars indicate the position of the $C_{ro}t_{1/2}$ of the two components of the ovary cDNA curve. The arrow indicates the $C_{ro}t_{1/2}$ of the globin cDNA reaction. The dotted line is an ideal first-order curve (see Fig. 2). (*B*) Tadpole globin cDNA. The dotted line is drawn through the data points and is not a first-order curve. □, Ovary cDNA; ■, globin cDNA; △, total yeast RNA.

most complex class contains approximately 18,000 sequences (M. M. Rosbash and S. M. Perlman, unpublished data). The $C_{ro}t_{1/2}$ values, which correspond to the midpoint of the hybridization of each class, are indicated in Fig. 3*A*. The adult globin cDNA formed a hybrid with ovary RNA with a $C_{ro}t_{1/2}$ no more than a factor of 3 greater than the $C_{ro}t_{1/2}$ of the most complex class of ovary RNA. This indicates that the concentration of adult globin sequence in the ovary RNA preparation is similar to that of the 18,000 different sequences present in the most complex class. Fig. 3*B* shows the same experiment performed with tadpole cDNA probe. The majority of the probe had hybridized by the highest $C_{ro}t$ values shown, indicating that the tadpole globin sequence was also present in the ovary RNA preparation. Furthermore, the rate at which the tadpole probe was rendered double stranded, although somewhat anomalous (see *Discussion*), was not markedly different from the rate at which the adult globin cDNA probe hybridized in Fig. 3*A*. Control experiments showed that incubation of the same probes with yeast RNA yielded no double-stranded cDNA (Fig. 3*B*).

Assay of Mature Oocyte RNA for the Tadpole and Adult Sequences. The mature oocyte contains a smaller percentage of poly(A)-containing RNA than does unfractionated ovary RNA (2), and preliminary experiments showed that it would be difficult to reach sufficiently high $C_{ro}t_e$ values to hybridize the globin cDNA with total mature oocyte RNA. Therefore, isolated poly(A)-containing RNA from mature oocytes was assayed for the globin sequences. The results, shown in Fig. 4, demonstrated that the globin sequence was also present in mature oocyte poly(A)-containing RNA. The rate of hybridization of the adult globin cDNA was at least one-fifth that of the most complex class of oocyte poly(A)-containing RNA, suggesting a maximum of a 5-fold difference in concentration. This is less than the value obtained in Fig. 3 and suggests that the concentration of adult globin sequence may be diminished in mature oocyte poly(A)-containing RNA as compared to total oocyte RNA. The tadpole cDNA was also rendered double stranded by hybridization with mature oocyte poly(A)-containing RNA (Fig. 4*B*). The data suggest that, like total ovary RNA, mature oocyte poly(A)-containing RNA contains less tadpole globin sequence than adult globin sequences. The lack of crossreaction between the adult and tadpole sequences and the lack of tadpole globin sequence in the adult globin RNA preparation (Fig. 2) indicate that the presence of the tadpole globin sequence in oocyte RNA is not due to erythroid cell contamination of the oocyte preparation. In order to exclude other somatic cells as the source of the globin sequences, poly(A)-containing RNA prepared from unfertilized ovulated eggs was assayed for the adult globin sequences (Fig. 5). The presence of these sequences in ovulated eggs reinforces the conclusion that they are present in germ cell RNA. The globin sequences are present in egg poly(A)-containing RNA at a somewhat reduced concentration (one-half to one-third) relative to mature oocyte poly(A)-containing RNA.

Assay of Swimming Tadpole RNA for Adult and Tadpole Sequences. Within 2 days of fertilization, the circulatory system of the developing embryo begins to function (8). The organism presumably contains a large amount of hemoglobin and it was therefore expected that the embryo would contain an increased amount of tadpole globin mRNA.

Consequently, cytoplasmic poly(A)-containing RNA prepared from stage 41 swimming tadpoles (8) was hybridized with globin cDNA (Fig. 6). As in Figs. 3 and 5, the hybridization of cDNA transcribed from the driver RNA is included. The results of the homologous hybridization experiments indicate that a major distinction between tadpole poly(A)-containing RNA and mature oocyte poly(A)-containing RNA is the presence of an

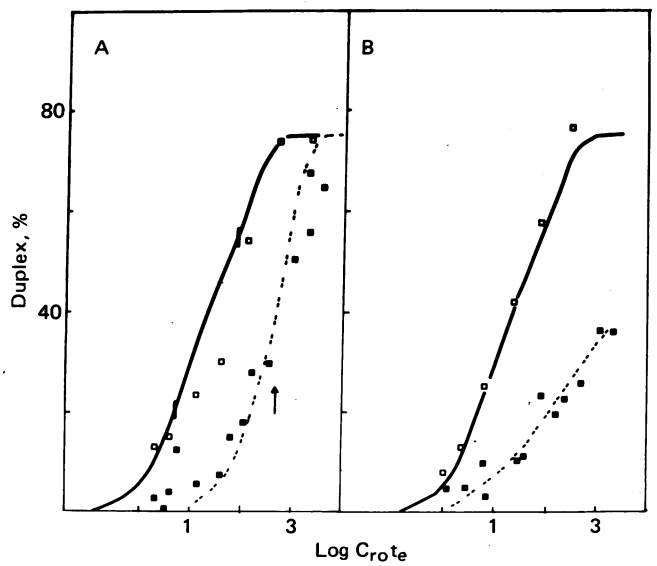

FIG. 4. Hybridization of adult and tadpole cDNA with oocyte poly(A)-containing RNA. Poly(A)-containing RNA was prepared from mature oocytes as described in *Materials and Methods*. The RNA was hybridized with ovary [^{3}H]cDNA and globin [^{32}P]cDNA. The ratio of poly(A)-containing RNA to globin cDNA was 4 to 7 × 10^5. The number and abundance of poly(A)-containing RNA sequences are identical in the ovary and mature oocyte (S. M. Perlman and M. M. Rosbash, unpublished data); for convenience, ovary cDNA was used in this experiment. (*A*) Adult globin cDNA. (*B*) Tadpole globin cDNA. Symbols are as in Fig. 3.

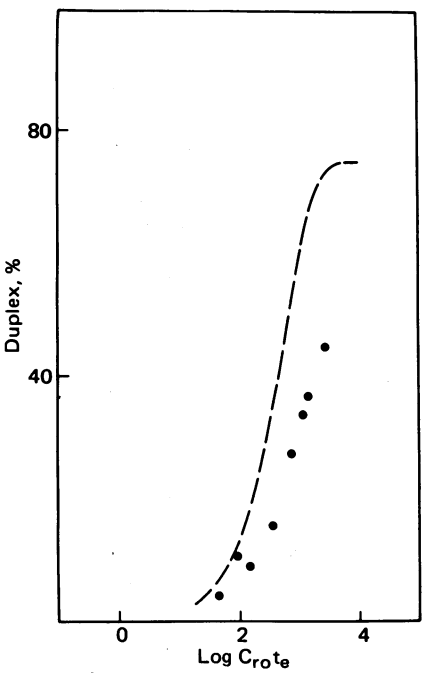

FIG. 5. Assay of egg poly(A)-containing RNA for adult globin sequences. Poly(A)-containing RNA was prepared from unfertilized ovulated eggs as described in *Materials and Methods*. The RNA was hybridized with adult globin [^{32}P]cDNA. The ratio of poly(A)-containing RNA to cDNA was 4 to 7 × 10^5. ●, Hybridization of egg poly(A)-containing RNA to adult globin cDNA; – –, annealing of mature oocyte poly(A)-containing RNA to adult globin cDNA, reproduced from Fig. 4*A*.

abundant component in the former that is absent in the latter. These data show that after 3 days of development the tadpole globin RNA sequence was an abundant sequence. The rate of hybridization of this cDNA was approximately the same as the most abundant, least complex class of the tadpole homologous hybridization curve. The intracellular concentration of the tadpole globin sequence was increased at least 300-fold relative to its concentration in the mature oocyte or in the unfertilized egg. In contrast, the concentration of the adult globin sequence had changed relatively little during the same period of development. Indeed, the hybridization of adult globin cDNA to tadpole poly(A)-containing RNA was well described by the hybridization curve of adult globin cDNA to mature oocyte poly(A)-containing RNA. Our conclusion from these data is that the adult globin sequence remains relatively unchanged in concentration during the first 3 days of development whereas the tadpole globin sequence is a major transcription product and increases in concentration at least 300-fold during the same time period.

DISCUSSION

In this paper, it is shown that *Xenopus* oocytes contain the RNA sequences for both adult and tadpole globins. The sequences were detected by hybridization of ovary RNA to cDNAs transcribed from the globin RNAs. The complexity of these cDNA probes is consistent with the presence of a low number (two to four) of 10S template RNAs in each globin RNA preparation. When hybridized to a vast excess of *Xenopus* DNA, the probes annealed with second-order kinetics and with a rate appropriate for the products of unique genes (data not shown) (12). In addition, the melting temperature of the hybrid formed between the ovary RNA and the globin cDNA was assayed and, within experimental error, was the same as the melting temperature of authentic 10S globin RNA·cDNA hybrids (data not shown). Both experiments strongly support the conclusion that the sequences detected by hybridization are identical to the globin structural gene sequences.

Two biological controls were performed to rule out contamination with non-oocyte RNA as the source of the globin RNA sequences. First, the oocyte RNA was assayed for the presence of tadpole globin RNA as well as adult globin RNA. This tadpole sequence shares no homology with the adult sequence under the conditions of hybridization and assay used in this study; in addition, the data in Fig. 2 indicate that the preparation of adult globin RNA contains less than 1% tadpole sequence. Because the tadpole globin sequence is present in oocyte RNA at a concentration somewhat less than but similar to that of the adult sequence, we conclude that these results cannot be explained by contamination of the oocyte preparation with blood cells which are expected to contain only the adult sequences. Second, adult globin RNA was also found in unfertilized egg RNA (Fig. 5). Ovulated eggs are not associated with follicle cells and we therefore conclude that the adult globin sequence is indeed present in *Xenopus* oocytes at approximately the concentration indicated.

The abundance classes of *Xenopus* ovary poly(A)-containing RNA have been carefully analyzed. These hybridization data, in conjunction with previously published data on ovary poly(A)-containing RNA (2), indicate that each oocyte contains approximately 1 million copies of approximately 18,000 different poly(A)-containing sequences. The hybridization of adult globin cDNA to ovary poly(A)-containing RNA indicates that the globin sequences are present at a concentration of approximately one-third to one-fifth the concentration of the

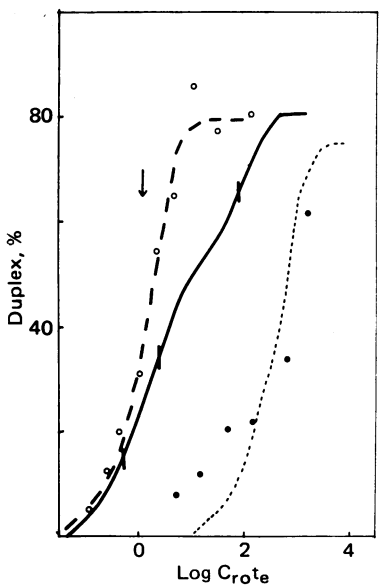

FIG. 6. Assay of stage 41 tadpole RNA for globin sequences. Poly(A)-containing RNA was prepared from stage 41 tadpoles as described in *Materials and Methods*. The RNA was hybridized with tadpole globin [^{3}H]cDNA and adult globin [^{32}P]cDNA. The ratio of poly(A)-containing RNA to tadpole globin cDNA was 2 × 10^4 and that of the RNA to adult globin cDNA was 4 × 10^5. The solid line indicates the hybridization of poly(A)-containing tadpole RNA with cDNA copied from this RNA. This three-component curve has been fitted to the data obtained in several experiments. The vertical bars indicate the $C_ot_{1/2}$ of the components of the tadpole RNA·cDNA hybridization. The arrow indicates the $C_ot_{1/2}$ of the tadpole RNA·tadpole globin cDNA reaction. – –, First-order curve indicating the hybridization of the tadpole globin cDNA to tadpole RNA; - - -, hybridization of mature oocyte RNA to adult globin cDNA (reproduced from Fig. 4*A* for ease of comparison); ●, adult globin cDNA; ○, tadpole globin cDNA.

18,000 members of the most complex class. On the basis of these calculations, we conclude that each *Xenopus* oocyte contains approximately 200,000–300,000 copies of the globin structural gene sequence.

The hybridization of tadpole globin cDNA to oocyte RNA is more difficult to interpret. It is not clear why the rate and extent of hybridization of tadpole globin cDNA to oocyte RNA is somewhat less than the comparable experiment with adult globin cDNA. One interpretation is that the concentration of the tadpole sequence in the oocyte is sufficiently less than that of the adult sequence so that the reaction is no longer in adequate RNA excess. This would reduce the plateau achieved at high $C_{ro}t$ values and also eliminate the first-order character of the reactions. This is consistent with the data as seen most clearly in Fig. 4*B* and is consistent with model reconstruction experiments that suggest that the reactions with adult globin cDNA (Figs. 3*A* and 4*A*) are only marginally in RNA excess (S. M. Perlman and M. M. Rosbash, unpublished data).

The presence of at least 200,000 copies of the adult globin structural gene sequence in each mature oocyte is striking. It has been previously reported that the globin genes are transcribed in nonerythroid cells (20). In these cells, the sequences are largely restricted to the nucleus. We have no information regarding the cellular location of the globin sequences in oocytes. However, their presence at a similar concentration in ovulated eggs (after germinal vesicle breakdown) suggests that these sequences are not necessarily restricted to the nucleus.

By 3 days after fertilization, the tadpole globin sequence is one of the major sequences of tadpole cytoplasmic poly(A)-containing RNA. The rate at which the tadpole globin cDNA is rendered double stranded suggests that each globin RNA sequence accounts for approximately 0.2–0.5% of the poly(A)-containing RNA of the tadpole. In contrast, the adult globin RNA is present at approximately the same concentration in the tadpole as in the mature oocyte. These data are similar to the results of Gurdon *et al.* (21) in which mouse globin mRNA, injected into embryos, persisted to the tadpole stage and may indicate that the adult globin RNA sequence is stable during embryogenesis.

The large number of globin sequences in oocytes suggests the presence of active transcription of globin DNA during oogenesis. This unexpected result can be interpreted in at least two different ways. It is possible that the globin sequence plays an as yet undetermined role in oogenesis or in early development. Alternatively, the lampbrush chromosomes may represent a system of promiscuous transcription in which most or all of the structural gene sequences utilized during the lifetime of the organism are transcribed at a high rate. We favor this latter interpretation.

We thank Drs. R. Old, B. Roberts, and D. Spector for helpful advice. We particularly thank Dr. U. Schibbler for describing to us the effects of thiourea and the procedures for isolating blood from tadpoles. We are grateful to Dr. J. Rosekrans and C. Blifeld for help with bleeding the tadpoles and to Dr. L. Hereford for a gift of yeast RNA and helpful discussions. We thank Dr. J. Beard and the National Cancer Institute for the generous gift of avian myeloblastosis virus reverse transcriptase. Preliminary experiments were performed in the laboratory of Dr. J. O. Bishop in Edinburgh, Scotland, and we thank him for support during this period. This work was supported by a grant from the National Institutes of Health to M.M.R. M.M.R. was also supported by a Career Development Award from the National Institutes of Health, S.M.P. by the Helen Hay Whitney Foundation, and P.J.F. by the Science Research Council (Britain) and North Atlantic Treaty Organization.

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