Nuclear Exclusion of Transcription Factor IIIA and the 42S Particle Transfer RNA-binding Protein in *Xenopus* Oocytes: A Possible Mechanism for Gene Control?

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ABSTRACT The intracellular location of 7S and 42S RNP particles in *Xenopus* oocytes has been determined by immunohistochemistry. Using antibodies directed against the 48-mol-wt protein component of the 42S particle and against transcription factor IIIA, the protein moiety of the 7S particle, we show that these ribonucleoprotein particles are detectable only in the oocyte cytoplasm, being excluded from the nucleus. The mechanism of this nuclear exclusion, and its possible significance in the regulation of 5S RNA gene expression, are discussed.

During the early stages of oogenesis in *Xenopus laevis*, 5S RNA and tRNA constitute ~75% of total oocyte RNA, while only a very small amount of ribosomal RNA is synthesized (1, 2). The two RNA species are not found free in the cytoplasm, but form part of two ribonucleoprotein (RNP) particles, the 7S (3) and the 42S[1] (2, 4) RNP particles. The 42S particle consists of tRNA, 5S RNA, and two proteins of 48,000 and 40,000 mol wt (5, 6). The larger of the two proteins binds to tRNA and the smaller to 5S RNA (5). The 7S particle contains one molecule of 5S RNA and one molecule of a 39,000-mol-wt protein (3), which has been shown by chemical and immunological criteria (7, 8) to be identical to transcription factor IIIA (TFIIIA), but different from the 40,000-mol-wt protein present in the 42S particles (7). TFIIIA is the protein whose binding to 5S RNA genes has been shown to be required for transcription of these genes (7, 9, 10), and may also play an important part in the developmental regulation of the *Xenopus* oocyte-type 5S genes (11, 12, 13). Brachet (14) showed that pyronin and other basic dyes specific for RNA stained the cytoplasm of previtellogenic oocytes more strongly than the nucleus. Since the bulk of the RNA in these oocytes is either 5S or tRNA, this has been taken to indicate that the 7S and 42S RNP particles are cytoplasmic (15). However, the intracellular location of the protein components of the particles has never been directly determined.

Recent experiments in which labeled RNA was injected into mature *Xenopus* oocytes (16) showed that transfer RNA (tRNA) is excluded from the oocyte nucleus while microinjected 5S RNA enters the nucleus where it becomes concentrated in the nucleoli, the sites of ribosome formation. To analyse further the nucleocytoplasmic transport of these RNP particles, we used antibodies against the protein components of the particles to determine their intracellular location. We find that both RNP particles are located in the oocyte cytoplasm, and in fact must be excluded from the nucleoplasm by some mechanism.

RESULTS AND DISCUSSION

42S RNP particle proteins were prepared on sucrose gradients by the method of Denis et al. (5) and separated by PAGE. Individual bands were recovered by electroelution (17) and used to immunize rabbits. One of the resulting antisera bound to a protein of 48,000 apparent molecular weight from previtellogenic *Xenopus* oocytes (Fig. 1*A*, lane *2*), and showed only very weak cross-reactivity with other protein bands. The antiserum bound to a protein of the same apparent molecular weight when sucrose gradient-purified 42S RNP particle proteins were used as antigen (data not shown). The anti-7S RNP particle antibody was a generous gift of Dr. D. Brown and has previously been characterised (7, 18) as binding to the protein component of 7S RNP particles, TFIIIA. Fig. 1*A*, lane *3*, shows that this antiserum binds strongly to a band of 39,000, the apparent molecular weight of TFIIIA (8). To prove that the antibodies also bound to 42S and 7S RNP particles, extracts from previtellogenic oocytes were bound to antibody immobilized on protein A-Sepharose beads. RNA was extracted from the immunoprecipitates and analysed by PAGE. As shown in Fig. 1*B*, lanes *3* and *4*, the anti-TFIIIA antiserum precipitated specifically 5S RNA, while the anti-42S 48,000-mol-wt protein (42S p48) precipitated both tRNA and 5S RNA. The 5S RNA contained in the 42S RNP particles is

[1] *Abbreviations used in this paper:* 42S p48, 42S 48,000-mol-wt protein; RNP, ribonucleoprotein; tRNA, transfer RNA; and TFIIIA, transcription factor IIIA.

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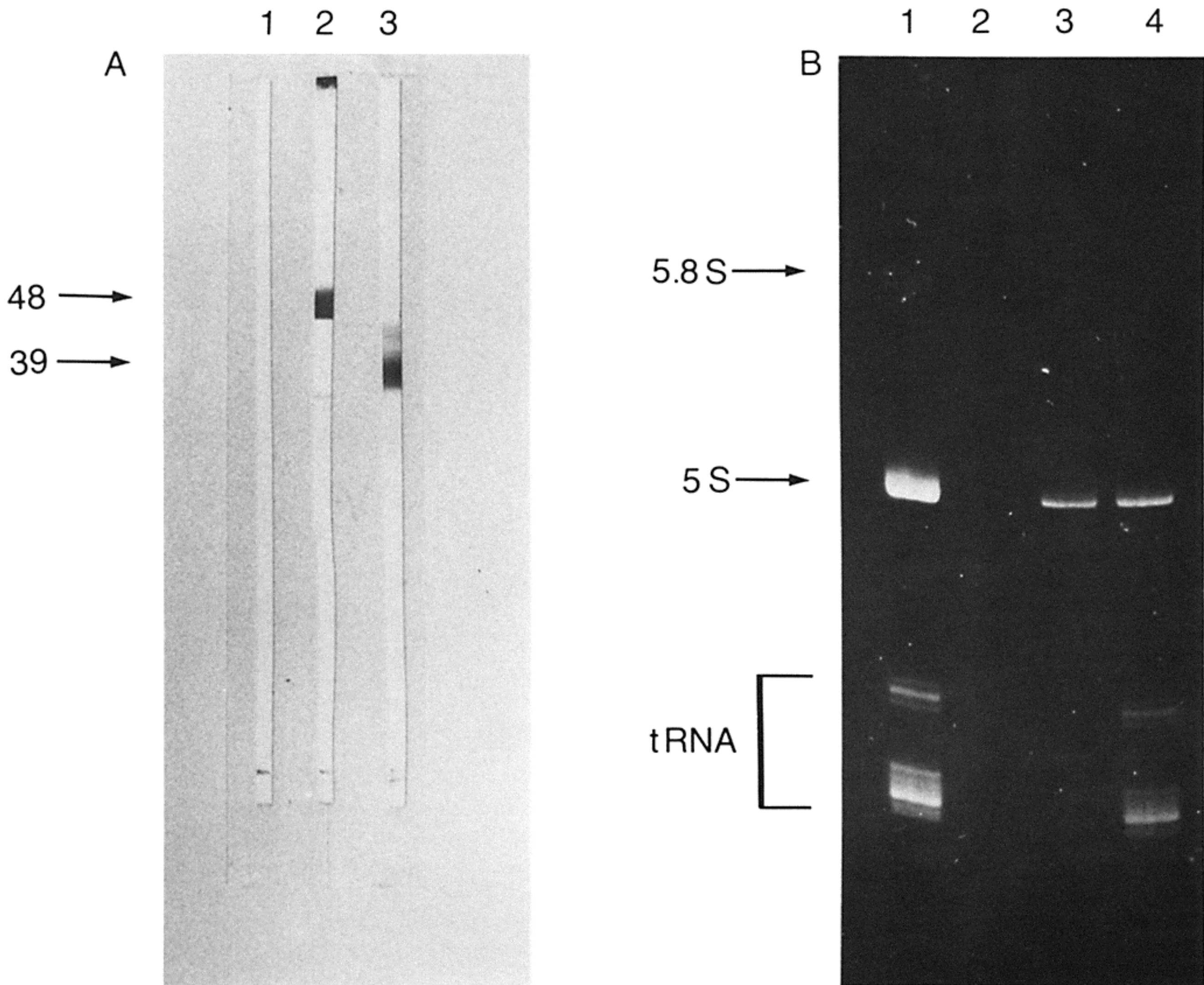

FIGURE 1 Characterisation of anti-TFIIIA and anti-42S p48 antibodies. (*A*) Immunoblot analysis of previtellogenic oocyte proteins. An ovary homogenate was prepared as described (5) and 1/10 was loaded on a 12% polyacrylamide gel (33). After electrophoresis the proteins were transferred to nitrocellulose (34) and visualized by binding first with antiserum then with horseradish peroxidase coupled to protein A, and finally staining with 4-chloronaphthol. All incubations and washes were done in Zeller's solution (32) (10 mM Tris-HCl pH 7.4, 100 mM $MgCl_2$, 0.5% Tween 20, 1% bovine serum albumin, 5% fetal calf serum). Lane *1*: preimmune serum. Lane *2*: anti-42S p48 antiserum: Lane *3*: anti-TFIIIA antiserum (a gift of Dr. D. D. Brown). Molecular weights ($\times 10^{-3}$) were determined by comparison with standards transferred from the same gel and stained on the nitrocellulose with amido black (34). (*B*) Immunoprecipitation of oocyte RNA. Homogenate from pre-vitellogenic oocytes was immunoprecipitated as described (16), except that antibody binding was carried out in 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$, and wash buffer contained 150 mM NaCl. These modifications were necessary because 42S and 7S RNP particles dissociate at higher salt concentrations. RNA was extracted and separated by 7 M urea–8% PAGE (16) and stained with 0.5 μg/ml ethidium bromide. Lane *1*: RNA extracted from homogenate before immunoprecipitation. Lane *2*: immunoprecipitated with pre-immune serum. Lane *3*: immunoprecipitated with anti-TFIIIA antiserum. Lane *4*: immunoprecipitated with anti-42S p48 antiserum.

thought to bind to the smaller 40,000-mol-wt protein component while tRNA binds to 42S p48 directly (5). Thus the antiserum presumably precipitates intact 42S particles. The relative abundance of 5S and 5.8S RNA in lane *1* of Fig. 1*B* demonstrates the very early developmental stage of the oocytes used in this experiment. The 5.8S, 18S, and 28S ribosomal RNA at this stage (Dumont stage 1 [19]) are present in a much lower molar ratio than either 5S or tRNA (1, 2), while fully grown *Xenopus* oocytes or somatic cells have a 1:1 ratio of 5.8S to 5S RNA (data not shown).

Previous workers (4, 6) have shown that the protein constituents of the 42S and 7S RNP particles are major components of previtellogenic oocytes. We reinvestigated this by comparing homogenates of previtellogenic oocytes containing known amounts of total protein (determined by the method of Lowry et al. [20]) and known amounts of bovine serum albumin by visual inspection and densitometry of Coomassie Brilliant Blue-stained one-dimensional polyacrylamide gels (data not shown). We estimate that 42S p48 constitutes 15–20% and 42S p40 and TFIIIA 8–10% each of total soluble previtellogenic oocyte protein. These estimates must be considered with care due to potential sources of error. For example, the oocyte proteins may react differently than bovine serum albumin with either the Folin reagent or Coomassie

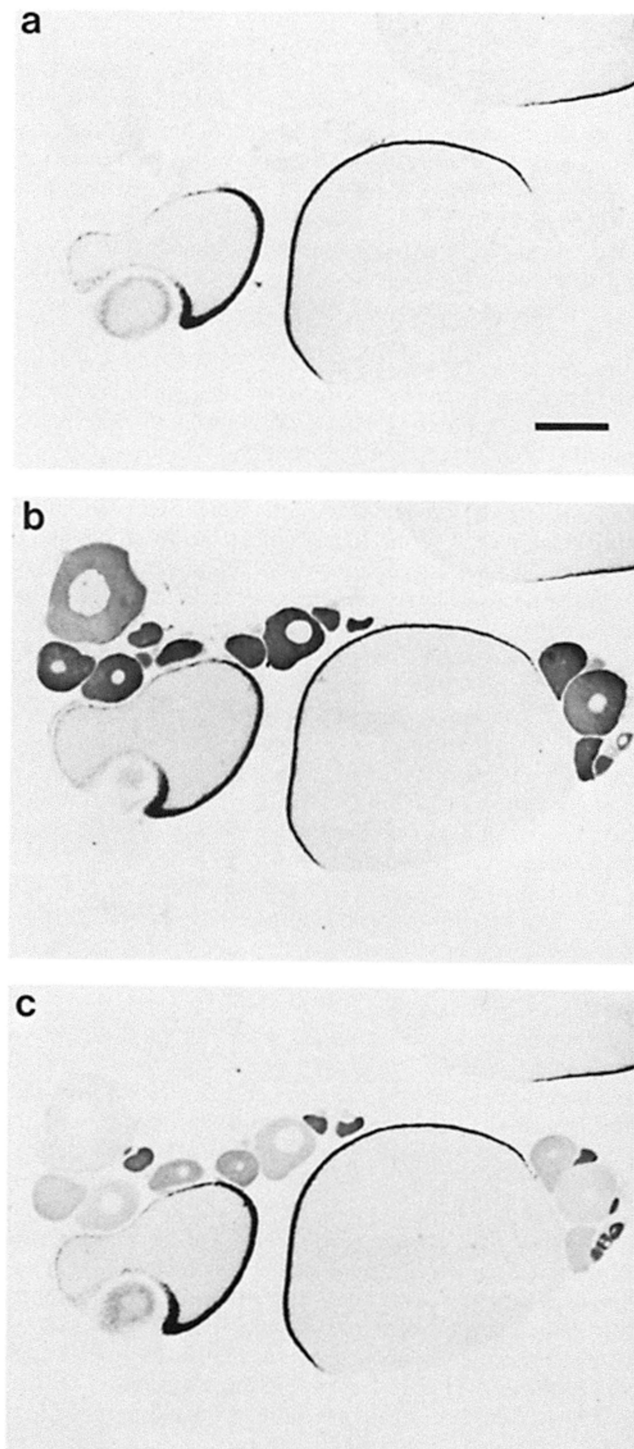

FIGURE 2 Immunolocalisation of 42S p48 and TFIIIA on serial ovary sections. Mature *X. laevis* ovary sections were fixed by freeze-substitution in ethanol and embedded in wax. 8-μm sections were taken and incubated with rabbit antiserum diluted 1:20 in Zeller's solution (10 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 0.5% Tween 20, 1% bovine serum albumin, and 5% fetal calf serum). After washing in Zeller's solution, the sections were incubated with protein A-peroxidase (New England Nuclear, Boston, MA), washed, and stained by incubation with 3-amino-9-ethyl carbazole (Sigma Chemical Co., St. Louis, MO) and hydrogen peroxide. Full details of the methods are given in Zeller et al. (32). Staining with (a) Anti-42S p48 antiserum, (*b*) Anti-TFIIIA antiserum, and (c) Pre-immune serum. Bar, 200 μm. × 50.

Brilliant Blue, or other abundant proteins may comigrate with the proteins under study in the one-dimensional gels. Nevertheless, it is clear that the 7S and 42S RNP proteins are present in massive amounts in early oogenesis.

Fig. 2 shows the intracellular location of the 42S and 7S RNP particles on sections of *Xenopus* oocytes as determined using the anti-42S p48 and anti-TFIIIA antibodies. Both antibodies stain exclusively the cytoplasm of previtellogenic and early vitellogenic oocytes (stage I, 50–300 μm diameter; stage II, 300–450 μm diameter; and stage III, 450–600-μm diameter; fully grown oocytes, stage VI, are 1,200 μm in diameter) (19). We are unable to detect any significant staining in the nucleus. Although the staining method is qualitative rather than quantitative, it is also apparent that 42S p48 concentration reaches a maximum in the earliest stage-I oocytes seen on the section and decreases gradually through late stage I, stage II, and stage III and is no longer detectable in larger oocytes. TFIIIA concentration, in contrast, remains comparatively constant until a later stage. When yolk deposition starts at late stage II, TFIIIA concentration decreases markedly until it is no longer detectable by our immunological method after stage IV (Fig. 2 *b*). The onset of vitellogenesis is accompanied by rapid growth. Our experiments do not distinguish whether the decreases in concentration we see are due solely to an increase in oocyte volume or to a decrease in the total quantity of TFIIIA or 42S p48 per oocyte. Using immunological procedures, Roeder et al. (21) have shown that the quantity of TFIIIA per oocyte remains constant through oocyte stages I–V before decreasing 20–30-fold at stage VI. Similarly, 42S p48 was detectable by gel electrophoresis of fractionated homogenates of oocytes in stages I and II, but not in stage VI (6). Since the decreases in concentration we see occur largely in stage-I, stage-II and stage-III oocytes, they are probably therefore mainly due to dilution of the RNP brought about by an increase in oocyte volume.

The cytoplasmically located proteins detected by the antibodies most probably represent the distribution of the corresponding RNP, and thus we asked whether during oocyte development one might not find a time at which the proteins are nuclear, before 5S and tRNA accumulation takes place. Fig. 3 shows that TFIIIA and 42S p48 are located in the cytoplasm from the earliest stages at which they can be detected by our immunolocalization method (which is relatively insensitive). This was determined by staining sections through the ovaries of stage-63 (22) tadpoles, which are quite far advanced in metamorphosis but still have motile tails. Some of the oocytes (diameter 20–100 μm) in these very young ovaries are stained intensely in their cytoplasm by anti-42S p48 (Fig. 3 *b*) and anti TFIIIA antisera (Fig. 3 *c*), while other regions of the ovary, containing smaller oocytes, remain unstained. Staining with Heidenhain's ferric hematoxylin (Fig. 3 *d*) showed that the larger oocytes that react with the antisera contain multiple large nucleoli and thus have already undergone ribosomal DNA amplification, which takes place during pachytene (23–25) and before the oocytes start any substantial growth in diameter (ferric hematoxylin proved better than Hoechst 33258 for detecting nucleoli in our preparations). The oocytes that accumulate TFIIIA and 42S p48 have a basophilic cytoplasm (Fig. 3 *d*), presumably reflecting the accumulation of 5S and tRNA, and their nuclei are in diplotene and thus already have lampbrush chromosomes (23).

One possible explanation for the exclusively cytoplasmic

location of the 42S and 7S RNP particles is that they are attached to or are part of some cytoplasmic structure and are therefore unable to diffuse freely between cytoplasm and nucleus. It is known that 7S and 42S RNP particles are completely soluble in homogenates of previtellogenic oocytes (2, 3). We therefore microinjected such homogenates into fully grown oocytes, in which the endogenous concentration of TFIIIA and 42S p48 is not high enough to be detected (Fig. 2). The results from this experiment showed that microinjected 7S and 42S RNP particles diffuse uniformly throughout the cytoplasm of fully grown oocytes, but fail to enter the oocyte germinal vesicle even after 24 h of incubation (data not shown).

Our results show that 7S and 42S RNP particles are excluded to a significant extent from the nucleoplasm of *Xenopus* oocytes. The immunohistochemical technique used in the analysis is probably relatively insensitive, and thus one cannot say that oocyte nuclei do not contain any of the proteins, but rather only that they are present at much higher concentration in the cytoplasm. The mechanism of exclusion of these proteins from the nucleus is unknown, but one obvious possibility is that the nuclear membrane acts as a barrier that blocks the movement of the RNP particles into the nucleus. The vast majority of oocyte TFIIIA and 42S p48 are bound to RNA, forming 7S and 42S RNP particles (3, 6; our unpublished data), and thus our observations likely reflect the distribution of the RNP and not of the free proteins. On the other hand, at least in the case of TFIIIA, it is known that the oocyte nucleus does contain some protein molecules that were not detectable in our immunohistochemical assay. Indeed, the oocyte nucleus contains about 100,000 5S genes (27, 28), and in vitro transcription studies of oocyte chromatin (11, 12) suggest that a large proportion of these have bound TFIIIA. Furthermore, the in vitro transcription system of Birkenmeier et al. (29), which is extracted from manually isolated *Xenopus* oocyte nuclei, does contain TFIIIA, which is necessary for 5S gene transcription. Similar results regarding the cytoplasmic location and inability to detect nuclear staining of TFIIIA have been obtained by M. Schlissel and D. Brown (personal communication) using rhodamine conjugated goat anti-rabbit antibody. Using this presumably more sensitive method, they were still able to detect cytoplasmic TFIIIA in somewhat later oocyte stages.

TFIIIA is small enough (39,000 mol wt) to diffuse freely through nuclear pores whose diameter usually allows free passage of proteins of <68,000 mol wt (reviewed by Bonner [30]). The simplest explanation for the observed exclusion of the 7S RNP from the nucleoplasm is that it is a result of 5S RNA binding to TFIIIA. It is not yet known whether TFIIIA or 42S p48 would be able to enter the nucleoplasm in the free state. Experiments to test this are in progress. The exclusion of these RNP particles from the nucleoplasm is of relevance to two cellular events. First, during their studies on the control

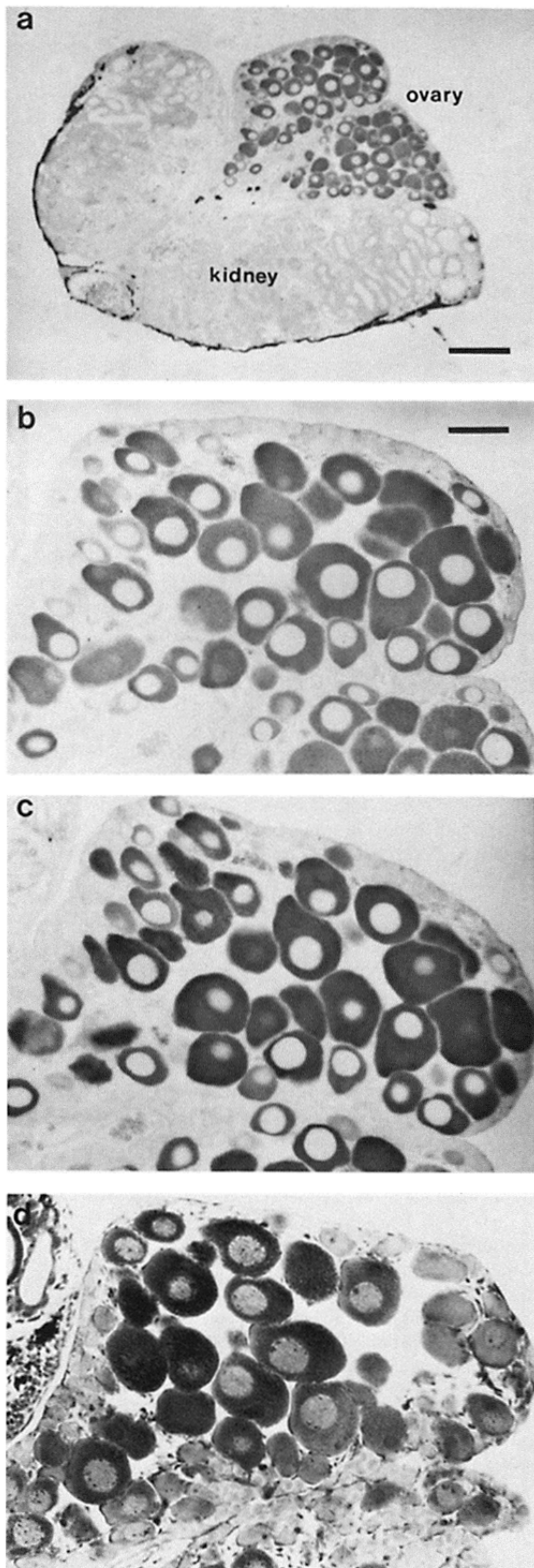

FIGURE 3 Immunolocalisation of 42S p48 and TFIIIA on serial sections of a tadpole ovary. Sections through the kidney and ovary of a stage-63 (19) *X. laevis* tadpole were stained as described in Fig. 2. (*a*) Section through kidney plus ovary stained with anti-42S p48 antiserum. Bar, 200 μm. × 50. (*b*) Ovary stained with anti-42S p48 antiserum. Bar, 70 μm. × 150. (*c*) Ovary stained with anti-TFIIIA antiserum. (*d*) Section of the same ovary stained with Heidenhain's ferric hematoxylin and eosin according to standard histological technique. The black border round the margin of the kidney is formed by pigment-containing cells.

of expression of 5S genes, Pelham and Brown (7) showed that 5S RNA in excess could inhibit in vitro transcription of 5S genes, presumably by competing for binding to TFIIIA, and suggested that this might provide a mechanism of feedback inhibition of 5S RNA synthesis. If 5S RNA binding to TFIIIA results in the separation of TFIIIA and the 5S genes into different cellular compartments, the cytoplasmic location of 5S RNA-bound TFIIIA could play an important role in the control of 5S gene expression. Even before the nucleocytoplasmic distribution of TFIIIA was known, Honda and Roeder (8) and Dixon and Ford (31) advanced the idea that nucleocytoplasmic transport could be involved in 5S gene control. Our observations provide experimental support for this view.

Second, the exclusion of these RNP particles is in marked contrast to the nucleocytoplasmic location and movement of another type of RNP, a U-rich small nuclear RNA particle. Complete U-snRNP particles are located in the nucleus of previtellogenic oocytes and somatic cells. Mature oocytes, however, accumulate substantial amounts of the snRNA-binding proteins in a particle devoid of snRNA, which is excluded from the nucleus (32). Upon injection of exogenous snRNA, the snRNP-binding proteins move into the nucleus, indicating that in contrast to the 7S particle the proteins on their own are cytoplasmic while the RNP has nuclear affinity. It will be interesting to see whether RNA binding has a general role in the nucleocytoplasmic transport of macromolecules.

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