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Related 5S RNA transcription factors in *Xenopus* oocytes and somatic cells

(cell-free transcription/antibodies/ribonucleoprotein particles/feedback regulation of transcription)

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ABSTRACT *Xenopus* oocytes contain an abundant protein that binds specifically to the center of 5S RNA genes and directs their transcription by RNA polymerase III. This protein also binds to 5S RNA. We show here that transcription of cloned 5S RNA genes in extracts derived from *Xenopus* tissue culture cells is dependent on the intragenic control region and is inhibited by 5S RNA and by antibodies raised against the previously characterized oocyte transcription factor. Somatic cells contain a protein that is similar to the oocyte factor in charge, affinity for heparin-agarose, and antigenicity but has an apparent molecular mass about 2000 daltons greater than that of the oocyte protein. Our experiments strongly suggest that this larger protein is the transcription factor for 5S RNA genes in somatic cells. The 5S RNA may regulate its own synthesis in somatic cells by binding to this protein, which is present at a low concentration. The presence of two different proteins responsible for 5S RNA synthesis in oocytes and in somatic cells cannot by itself explain the developmental control of oocyte and somatic 5S RNA genes, because somatic cell extracts transcribe both types of gene.

Transcription of 5S RNA genes in extracts of *Xenopus* oocytes is dependent on an intact intragenic control region (1, 2), which is recognized by a specific transcription factor (3, 4). We have recently shown that this factor can also bind to 5S RNA, so that added 5S RNA specifically inhibits transcription of 5S DNA (4). In addition to this protein's role as a transcription factor, the interaction of this single protein with the gene and the gene product (5S RNA) might be involved in three very different biological roles. First, the protein binds to 5S RNA in immature oocytes, forming an abundant 7S particle whose function is to store 5S RNA for subsequent assembly into ribosomes. Second, 5S RNA could regulate its own synthesis by binding to the protein and inhibiting transcription of 5S RNA genes. Third, the protein could be responsible for the activation of oocyte-specific 5S RNA genes during oogenesis.

The first of these roles, the storage function in early oocytes, has been demonstrated in early oocytes (4–6). In this paper we have addressed the second and third putative roles. Feedback inhibition might be expected to occur in somatic cells, where 5S RNA accumulation is coupled to ribosome assembly. We show here that 5S RNA can specifically inhibit its own synthesis in extracts prepared from somatic cells. These extracts have extremely low levels of the oocyte transcription factor. However, they contain a different protein that crossreacts antigenically with the oocyte factor and has properties similar to it. We also present evidence that the relative abundance of these two related proteins cannot by itself account for the differential expression of somatic and oocyte-specific 5S RNA genes.

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MATERIALS AND METHODS

Plasmid DNAs. Recombinant plasmids containing single inserts of *Xenopus laevis* oocyte 5S RNA gene (Xlo), pseudogene, or trace oocyte gene (Xlt) cloned in pBR322 were constructed. A single repeating unit of *X. borealis* somatic 5S DNA (pXbs1) and deletion mutants derived from this gene have been described (1, 2, 7). A cloned *Drosophila* arginine tRNA gene was obtained from D. Söll (8).

Preparation of Cellular Extracts. Extracts of *X. laevis* kidney-derived tissue-culture cells were prepared by the method of Manley *et al.* (9), with the following modification. The final $(NH_4)_2SO_4$ precipitate was redissolved in one packed-cell volume of J buffer [7 mM $MgCl_2$/70 mM NH_4Cl/0.1 mM EDTA/10 mM Hepes, pH 7.5/2.5 mM dithiothreitol/6% (vol/vol) glycerol]. The extract was dialyzed for 12 hr against 100 vol of J buffer, centrifuged to remove insoluble material, and frozen at −70°C. Extracts from staged embryos were prepared similarly. Liver was homogenized in J buffer containing 25% sucrose, treated with 10% saturated $(NH_4)_2SO_4$, and centrifuged at 100,000 × *g* (9). The supernate was diluted 1:3 for immunoprecipitation or chromatography on heparin-Sepharose (Pharmacia).

Transcription Reactions. These reactions were carried out at 23°C for 1 hr in J buffer containing 0.02 mM [α-^{32}P]GTP (Amersham, final specific activity 10–40 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels), each unlabeled nucleoside triphosphate at 0.2 mM, recombinant plasmid DNA at 4–5 μg/ml, and 50% by volume of the somatic cell extract or oocyte nuclear extract (10). Products were analyzed on polyacrylamide gels containing 7 M urea (1).

Preparation of Antiserum. A lightly stained $NaDodSO_4$ gel band containing 75 μg purified oocyte transcription factor (4) was excised and homogenized with 1 ml phosphate-buffered saline (P_i/NaCl) and 1 ml of complete Freund's adjuvant. The mixture was injected into a rabbit, 0.6 ml in each thigh muscle and the rest in 0.2-ml portions subcutaneously. Four weeks later 100 μg of the purified protein (not cut from a $NaDodSO_4$ gel) was administered similarly in incomplete Freund's adjuvant. The rabbit was bled at weekly intervals. IgG was precipitated from the serum with 33% saturated $(NH_4)_2SO_4$. The precipitate was redissolved in 17 mM sodium phosphate buffer at pH 6.6, passed through a Sephadex G-50 column and then a DEAE-cellulose column in this buffer, adjusted to 0.1 M KCl, and passed through a heparin-Sepharose column to remove residual ribonuclease. Purified IgG was added to transcription reactions at 0.1–0.4 mg/ml. Coupling to CNBr-activated Sepharose (Sigma) was performed as recommended by Pharmacia.

Abbreviation: P_i/NaCl, phosphate-buffered saline.

Detection of Antibody-Binding Proteins in Cellular Extracts. Proteins were separated either on $NaDodSO_4$ gels or on 10% polyacrylamide gels containing 8 M urea and 5% (vol/vol) acetic acid (11). For the latter, the proteins were first precipitated with 8% trichloroacetic acid and redissolved in 8 M urea/5% acetic acid. Proteins were transferred electrophoretically from the gels to nitrocellulose filters as described by Towbin *et al.* (12). The filters were incubated for 1 hr in P_i/NaCl containing 1% hemoglobin and 2% bovine serum albumin, then for 8 hr in the same solution containing crude antiserum at 20 μl/ml, washed with five changes of P_i/NaCl, and incubated for a further 2–8 hr in P_i/NaCl/hemoglobin/bovine serum albumin containing ^{125}I-labeled staphylococcal protein A (New England Nuclear) at 0.3–1.0 μCi/ml. After washing with 5 changes of P_i/NaCl, the filters were blotted dry and autoradiographed at −70°C with intensifying screens.

RESULTS

Transcription of 5S DNA in a Somatic Extract Is Directed by the Intragenic Control Region. We prepared extracts from a kidney-derived *Xenopus* cell line, using a modification of the high-salt extraction procedure described by Manley *et al.* (9). Such extracts faithfully transcribe 5S RNA and tRNA genes (Figs. 1–3), although the efficiency of transcription of 5S RNA genes (about 0.75 transcripts per gene per hr) is about 1/20th of that in the oocyte nuclear extract we have used previously (10). 5S RNA genes that have undergone a deletion from the 5′ side to residue 47 also direct accurate transcription in this extract (Fig. 1). Genes with deletions from the 3′ side as far as residue 83 and with the residual DNA linked to the last 15 residues of the gene (to provide a termination site) yielded transcription products of the expected size. The relative abundance and sizes of the products shown in Fig. 1 are similar to those obtained in oocyte extracts (1). We conclude that the same intragenic control region, between residues 50 and 83, is responsible for transcription in both oocyte and somatic systems. Fig. 1 (tracks 9–11) also shows that cloned oocyte-type 5S RNA genes are transcribed in the somatic cell extract, even though no endogenous synthesis of oocyte-type RNA could be detected with this cell line (data not shown).

5S RNA Inhibits Its Own Synthesis in the Somatic Cell Extract. Fig. 2 shows the effect of added RNA on transcription in the somatic cell extract. At 10 μg/ml, 5S RNA inhibited transcription of 5S DNA by almost 90% without significantly affecting tRNA synthesis. The 5S RNA used in this experiment was prepared from ovary, but similar results were obtained with 5S RNA from liver. In contrast, added *Xenopus* tRNA had only a slight nonspecific inhibitory effect. These and the previous results suggest that somatic cells contain a 5S RNA transcription factor with properties similar to those of the oocyte factor.

Antibodies to the Oocyte Transcription Factor Inhibit 5S RNA Synthesis in the Somatic Extract. We raised antisera to highly purified oocyte transcription factor and tested the effects of the IgG fraction in both the oocyte and somatic cell-free systems. Fig. 3A shows that the immune IgG inhibited 5S RNA synthesis in the oocyte extract by about 95%. Transcription of tRNA genes in the same reaction mixture was slightly stimulated, probably because the 5S RNA genes were no longer competing for common transcription factor(s). Nonimmune IgG had

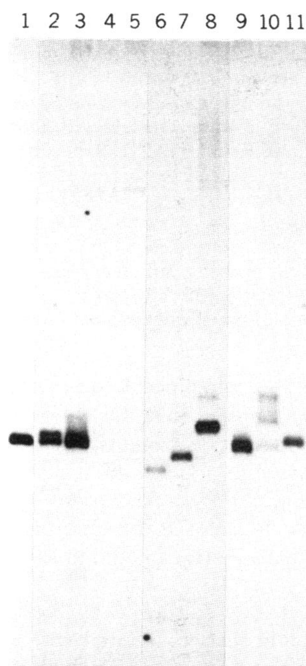

FIG. 1. Transcription in the somatic cell extract of intact 5S RNA genes and those with deletions. An autoradiogram of a polyacrylamide gel is shown. The templates were as follows: Track 1, *X. borealis* somatic gene (pXbs1); tracks 2–5, Xbs1 with deletions from the 5′ side into the gene, the deletions ending at residues +28 (track 2), +47 (track 3), +61 (track 4), and +74 (track 5); tracks 6–8, Xbs1 with deletions extending from the 3′ side into the gene, the residual DNA fragment being linked to the last 15 residues of the gene and the 3′ flanking sequence via a decameric synthetic linker, the deletions ending at residue +83 (track 6), +87 (track 7), and +97 (track 8); track 9, *X. laevis* oocyte (Xlo) pseudogene; track 10, Xlo gene; track 11, *X. laevis* trace oocyte gene (Xlt).

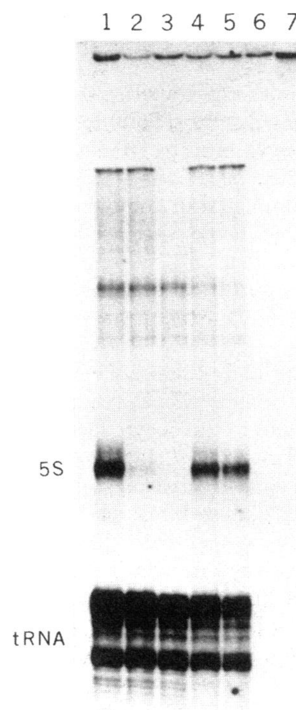

FIG. 2. Effect of 5S RNA and tRNA on transcription in the somatic cell extract. Reaction mixtures contained both cloned 5S DNA (Xbs) and a cloned tRNA gene. Track 1, control; track 2, 5S RNA added at 10 μg/ml; track 3, 5S RNA added at 20 μg/ml; track 4, tRNA added at 10 μg/ml; track 5, tRNA added at 20 μg/ml; track 6, no DNA; track 7, no DNA, 5S RNA and tRNA added at 20 μg/ml each.

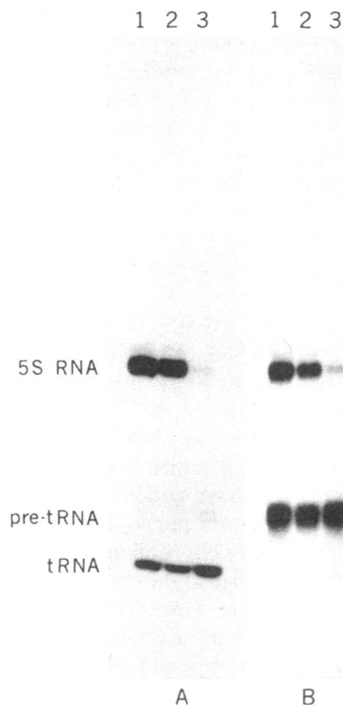

FIG. 3. Effect of antibodies on transcription. Transcription in the oocyte extract (*A*) or the somatic cell extract (*B*) was performed with a mixture of cloned 5S DNA (Xbs) and a cloned tRNA gene. Tracks 1, controls; tracks 2, nonimmune IgG; tracks 3, immune IgG.

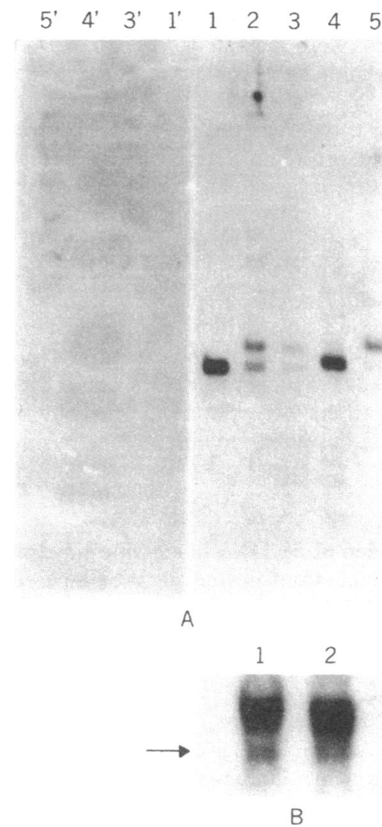

FIG. 4. Detection of antibody-binding proteins in cell extracts. Proteins were separated by electrophoresis in an acid/urea gel, transferred to a nitrocellulose filter, and incubated with antiserum and ^{125}I-labeled protein A. An autoradiogram of the nitrocellulose filter is shown. (*A*) Tracks contained manually isolated oocyte nuclei or high-salt extracts. Tracks 1–5 were screened with immune serum, tracks 1′–5′ with nonimmune serum. Track 1, 2 mature oocyte nuclei; track 2, 0.4 oocyte nucleus (0.4 μl of extract) plus extract from 10^6 tissue culture cells (20 μl of extract); track 3, extract from 3 stage 42 embryos (swimming tadpoles); track 4, extract from 30 unfertilized eggs; track 5, extract from 10^6 tissue culture cells (20 μl of extract). (*B*) Detail of a similar experiment, showing immunoprecipitates of high-salt extracts of tissue culture cells (track 1) and of adult liver (track 2). The arrow indicates the position of migration of oocyte-type factor.

no effect on transcription. These results confirm that 5S RNA synthesis in the oocyte system requires the transcription factor (see also Fig. 5*B*).

Fig. 3*B* shows a similar experiment with the cell-free system derived from somatic cells. In several experiments, immune IgG inhibited 5S RNA synthesis by 75–85%, while either stimulating or having no effect on tRNA synthesis. Nonimmune IgG sometimes slightly stimulated or inhibited both 5S RNA and tRNA synthesis, but had no consistent effect. From these experiments we conclude that 5S RNA synthesis in somatic cell extracts requires a protein that is antigenically similar to the oocyte transcription factor. Further evidence for this conclusion is presented below.

Identification of Antibody-Binding Proteins in Cellular Extracts. To identify the protein(s) in the somatic cell extract that react with the antibodies to the oocyte factor, we used the gel transfer technique of Towbin *et al.* (12). Proteins were separated by electrophoresis on an acid/urea gel and transferred electrophoretically to a nitrocellulose filter. The filter was incubated successively with antiserum and with ^{125}I-labeled staphylococcal protein A and then was autoradiographed. The results are shown in Fig. 4. Track 1 contained the extract from two mature oocyte nuclei. The antibodies recognized a single protein, which comigrated with the purified oocyte factor and did not react with nonimmune serum. By comparison with known amounts of the pure protein, we can estimate that this band contained approximately 4 ng of factor, or about 0.1% of the total protein loaded. The specificity of the antiserum is demonstrated by the absence of other bands in track 1. We could also detect oocyte factor in high-salt extracts from unfertilized eggs (track 4), although there was about 1/30th as much factor per egg as per oocyte nucleus.

Track 5 of Fig. 4 shows that the somatic cell extract contained a protein that reacted specifically with the antiserum but migrated more slowly than the oocyte factor did. The difference in migration persisted when oocyte and somatic extracts were mixed (track 2). Assuming that the antiserum reacts equally with the oocyte and somatic proteins, we can estimate that the somatic band represents about 0.001% of the protein loaded. Extracts of stage 42 embryos (swimming tadpoles) contained about as much oocyte factor as did unfertilized eggs, together with approximately twice this amount of somatic protein (track 3).

Extracts from tissue culture cells contained very small amounts of a protein that comigrated with the oocyte factor. This is seen more clearly in Fig. 4*B*, which shows part of a gel transfer experiment in which a larger volume of somatic extract was immunoprecipitated, and the precipitate was analyzed as in Fig. 4*A*. Also shown is a similar analysis of a high-salt extract of adult *Xenopus* liver. In both tissue culture cells and liver there is about 10-fold more somatic protein than putative oocyte factor.

From experiments such as that shown in Fig. 4*A*, the changes in the amounts of the immunoreactive proteins during oogenesis and embryogenesis can be estimated. The oocyte protein is most abundant in small oocytes. Mature oocytes have about 10% as much factor, and about 97% of this is lost during ovulation.

The residual oocyte-type protein is diluted out during embryogenesis, whereas the somatic protein gradually accumulates, so that swimming tadpoles contain more somatic than oocyte protein. However, stage 25 (tailbud stage) embryos that contain approximately 5-fold more oocyte than somatic protein synthesize only somatic 5S RNA (unpublished observations, to be described in detail elsewhere).

A Somatic Transcription Factor for 5S DNA. We have considered the possibility that there is sufficient oocyte-type transcription factor in the somatic extract to account for all the observed synthesis of 5S RNA. To investigate this, we compared the relative synthetic activity of the somatic and oocyte extracts with their content of oocyte-type factor. The experiment shown in Fig. 5*A* shows that addition of purified oocyte transcription factor to a somatic extract increased 5S RNA synthesis at least 10-fold, while causing a corresponding reduction in tRNA synthesis. The amount of factor added in this case was excessive, but because the factor is limiting in the reaction, the level of endogenous factor can in principle be estimated from the amount of added factor required to double the level of 5S RNA synthesis.

The assumption in such an experiment is that the added transcription factor has the same specific activity as the endogenous factor. However, in several experiments we have found the specific activity of our purified protein to be low and variable, apparently because of its tendency to aggregate. For this reason, we have used the oocyte nuclear extract as a source of factor that is highly active and very stable (10). As a control, the factor was removed from some of the oocyte extract by incubation with Sepharose beads to which antibodies had been covalently coupled. Fig. 5*B* shows that this treatment strongly and specifically reduced the ability of the oocyte extract to synthesize 5S RNA. Addition of purified oocyte factor restored this ability, showing that no other essential factors were removed by the treatment.

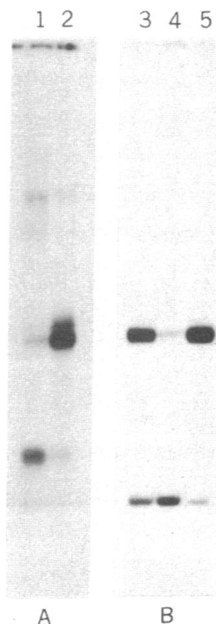

FIG. 5. Stimulation of transcription by purified oocyte factor. Transcription reaction mixtures contained both 5S DNA and tDNA. (*A*) Somatic cell extract, without (track 1) and with (track 2) 0.2 μg of purified oocyte factor. (*B*) Oocyte nuclear extract alone (track 3), treated with antibody-Sepharose to remove transcription factor (track 4), or treated with antibody-Sepharose and then supplemented with 0.2 μg of purified oocyte factor (track 5).

We then mixed small amounts of oocyte extract with the somatic extract and measured the effect on 5S RNA synthesis (Fig. 6). These amounts of oocyte extract did not cause any significant general stimulation of transcription, as shown by the lack of effect on transcription of tRNA genes, included as an internal control. Furthermore, oocyte extract that had been treated with antibody-Sepharose did not significantly stimulate 5S RNA synthesis when added to the somatic cell extract. In contrast, untreated oocyte extract specifically stimulated 5S RNA synthesis. This stimulation can be attributed to the transcription factor present in the oocyte extract, and it was proportional to the amount of oocyte extract added. By extrapolation to the baseline we can estimate that the endogenous factor activity in 10 μl of somatic extract corresponds to 0.35 μl of oocyte extract—i.e., the somatic extract contains about 1/30th as much factor per unit volume as the oocyte extract does. However, direct analysis of the protein in these extracts (Fig. 4) indicates that the somatic extract contains only about 1/300th as much (presumptive) oocyte-type factor as the oocyte extract does. If the small amount of putative oocyte-type factor in the somatic extract were the only active factor, the points in Fig. 6 should fall on the broken line. On the other hand, if the major band in the somatic extract (Fig. 4) is also a transcription factor, then the somatic extract would contain 1/30th as much factor as the oocyte extract does, in excellent agreement with the data in Fig. 6. These results suggest that the major protein detected in the somatic extract is a 5S RNA transcription factor.

The putative somatic factor migrates more slowly than the oocyte factor on both acid/urea and $NaDodSO_4$ gels, suggesting that it has a similar charge to the oocyte factor but is slightly larger. On $NaDodSO_4$ gels the oocyte factor comigrates with creatine kinase (40,000 daltons) (4); the somatic protein appears to be about 2000 daltons larger. Both proteins bind tightly to heparin-agarose, eluting between 0.5 and 1.0 M NaCl, as might be expected of nucleic acid binding proteins. We find no evidence that one of the proteins is derived from the other by

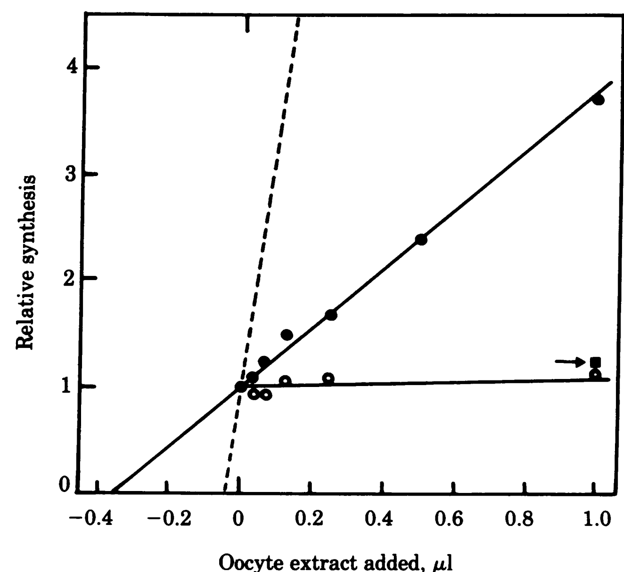

FIG. 6. Estimation of factor activity in a somatic cell extract. A mixture of 5S DNA and tDNA was transcribed in a 20-μl reaction volume containing 10 μl of somatic cell extract (the same batch of extract used in Figs. 4 and 5*A*) and serial dilutions of oocyte nuclear extract. The products were separated on a gel, and the amount of synthesis was estimated by cutting out the bands and measuring their radioactivities. ●, 5S RNA synthesis, oocyte extract added; ■, 5S RNA synthesis, antibody-Sepharose-treated oocyte extract added; ○, tRNA synthesis, oocyte extract added. The broken line is discussed in the text.

posttranslational modification. The somatic protein is stable during a 2-hr incubation of the somatic extract, and added oocyte protein does not have an altered mobility after such an incubation. Furthermore, the primary translation product obtained by immunoprecipitation of the *in vitro* translation products of ovary mRNA is the same size as the mature oocyte factor (unpublished observations). The oocyte and somatic proteins appear therefore to be similar but distinct gene products.

DISCUSSION

Immature *Xenopus* oocytes contain very large amounts of a protein that has at least two biological roles. It binds specifically to 5S RNA genes and directs their transcription by RNA polymerase III; it also binds to the 5S RNA that is made, forming a storage particle, which persists until the 5S RNA is incorporated into ribosomes (4–6). We have shown that antibodies raised against this protein will inhibit 5S RNA synthesis in extracts of somatic *Xenopus* cells, implying that an antigenically related protein acts as a transcription factor in these cells. The only such protein that we can detect in sufficient quantities to account for the observed transcription activity is clearly distinct from the oocyte factor, but has similar properties. The somatic factor appears to bind to the same intragenic control region as the oocyte factor does, because this region is required for transcription of 5S RNA genes in somatic cell extracts. Furthermore, because 5S RNA inhibits its own synthesis in these extracts at least as efficiently as in the oocyte extract, the somatic factor presumably can bind to 5S RNA. These properties of 5S RNA transcription factors may be quite general. Preliminary results indicate that transcription of 5S RNA genes in extracts of human KB cells is also directed by the internal control region and is specifically inhibited by added 5S RNA.

Our estimate of the minimal amount of factor (i.e., antibody-binding protein) in *Xenopus* liver and cultured somatic cells is of the order of 10^4 molecules per cell, compared with the 800 or so somatic 5S RNA genes that are transcribed in these cells (7). We suggest the following model: in somatic cells 5S RNA synthesis can be limited by feedback inhibition, caused by binding of the RNA to the transcription factor. This would provide a small pool of factor-bound 5S RNA that is available for incorporation into ribosomes. In growing *Xenopus* oocytes, on the other hand, 5S RNA synthesis is uncoupled from ribosome synthesis, allowing for the massive accumulation of RNA that is not incorporated into ribosomes until 1–2 months later (13). This uncoupling of 5S RNA synthesis compensates for the numerical imbalance between the 5S RNA genes and the 18S and 28S rRNA genes, which in oocytes are amplified in number (14). We suggest that this massive 5S RNA synthesis in the absence of ribosome formation is achieved by the synthesis of large amounts of transcription factor, which leads to an enormous expansion of the normal pool of factor-bound 5S RNA.

There are several possible explanations for why there should be different kinds of transcription factor in oocytes and somatic cells. The oocyte-specific factor may be adapted for its storage function in early oogenesis, or its gene may be adapted for a higher level of expression than its somatic counterpart. Alternatively, the oocyte and somatic factors might be derived from a single gene, using different initiation sites, termination sites, or splicing pathways.

Once its storage function is fulfilled, the oocyte factor is degraded. A previtellogenic oocyte contains at least 20 ng of the protein. Mature oocytes contain about 1/10th as much, but most of this is lost during ovulation, so that an unfertilized egg contains only about 0.1 ng of the factor (Fig. 4). However, this is still about 5 orders of magnitude more transcription factor than we estimate to be present in a tissue culture cell, so it is unlikely that complete destruction of the factor turns off 5S RNA synthesis at this stage, as has been suggested (3, 6). Liver cells may contain very small amounts of the oocyte protein, although it is possible that this is not in fact the same protein, but a degradation product or another minor form of somatic factor.

One major unexplained feature of 5S RNA synthesis concerns the types of RNA made in different cells. In oocytes the amount of 5S RNA that is synthesized is increased by the expression of one or more large families of 5S RNA genes (about 20,000 copies per haploid genome) that are not expressed in somatic cells (14). It is tempting to suggest that this specificity is related to the different transcription factors in the two types of cell. However, extracts of somatic cells can transcribe cloned 5S RNA genes of oocyte and somatic type with comparable efficiencies (Fig. 1), even though no synthesis of oocyte-type 5S RNA can be detected in the intact cells. This nonspecificity is not limited to transcription of cloned genes, because the oocyte-specific genes present in genomic DNA (obtained from erythrocytes) are also transcribed in somatic cell extracts (unpublished observations). Moreover, purified oocyte factor does not appear to be sufficient to activate the oocyte-type genes in somatic nuclei when these are injected into the nucleus of a mature oocyte (15). Last, early embryos synthesize only somatic 5S RNA even at stages when they contain predominantly oocyte-type factor (unpublished observations, to be described elsewhere). Thus, the existence of distinct transcription factors in oocytes and somatic cells cannot in itself explain the developmental control of the two classes of 5S RNA gene.

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