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Developmental Biology

A specific transcription factor that can bind either the 5S RNA gene or 5S RNA

(*Xenopus* oocytes/protection from DNase/ribonucleoprotein particles/developmental control of transcription)

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ABSTRACT **5S ribosomal RNA specifically inhibits transcription of cloned repeating units of 5S DNA in a nuclear extract of *Xenopus* oocytes. The inhibition can be explained by the interaction of 5S RNA with a transcription factor that binds specifically to a control region located within the 5S RNA gene. This transcription factor is identical to an abundant cytoplasmic protein that is known to be complexed with 5S RNA in immature *Xenopus* oocytes. Thus the presence of large amounts of this protein in these cells can account for both the high rate of synthesis and the subsequent storage of 5S RNA prior to ribosome synthesis.**

We are studying the control of transcription of 5S RNA genes, using an extract of *Xenopus laevis* oocyte nuclei and cloned *Xenopus* 5S RNA genes (1–3). In the course of these studies, we discovered that 5S RNA can specifically inhibit its own synthesis when added to this cell-free extract. Two additional findings have helped us explain the nature of this inhibition. First, analysis of deletion mutants of one cloned 5S RNA gene has identified a control region in the center of the gene that is necessary for accurate initiation of 5S RNA synthesis (2, 3). Engelke *et al.* (4) have isolated a protein from *Xenopus* ovaries that binds to this intragenic control region and appears to be required specifically for transcription of 5S DNA. Second, it is known that 5S RNA (and tRNA) synthesis occurs at a high rate in immature oocytes and precedes the formation of ribosomes (5–7). The 5S RNA accumulates and is stored in immature oocytes complexed with one or more nonribosomal proteins (8). In this report, we show that a major protein bound to 5S RNA in immature oocytes is, in fact, the transcription factor described by Engelke *et al.* (4). It appears that 5S RNA can inhibit its own synthesis *in vitro* by binding specifically to the very factor required for its synthesis.

MATERIALS AND METHODS

Preparation and Transcription of Cloned DNA. Plasmid pXbs1 contains a single repeating unit of *X. borealis* somatic 5S DNA in pBR322 (9). Deletion plasmids derived from this clone have been described (2, 3). pYH48, which contains a *Drosophila* arginine tRNA gene cloned in pBR322 (10), was obtained from D. Soll. pAd123, which contains a 1.8-kilobase insert including the VA RNA genes and flanking regions of adenovirus type 2 DNA in pBR322, was prepared by D. Bogenhagen. Preparation of DNA, transcription in the oocyte nuclear extract, and analysis of the products on polyacrylamide gels containing 7 M urea have been described (1–3). Transcription reaction mixtures contained the extract of one oocyte nucleus for each 2–4 μl of final reaction. The final buffer contained 7 mM $MgCl_2$, 70 mM NH_4Cl, 6% (vol/vol) glycerol, 10 mM Hepes at pH 7.5, 0.1 mM EDTA, 0.2 mM of each unlabeled nucleoside triphosphate, 0.02 mM [α-^{32}P]GTP or UTP (Amersham, final specific activity 10–40 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels), and DNA as indicated. All procedures involving use of recombinant DNA were carried out in accordance with the National Institutes of Health guidelines (P-2/EK-1).

Preparation of RNA and Ribonucleoprotein Particles. The 5S RNA was prepared from *X. borealis* ovaries (11). *Escherichia coli* "sRNA" was purchased from P-L Biochemicals. RNAs were electrophoresed on polyacrylamide gels, eluted, and further purified by chromatography on DEAE-cellulose. To prepare 7S and 42S particles, 10 ovaries from young *X. laevis* (3–5 cm long, purchased from Nasco, Fort Atkinson, WI) were homogenized in 50 mM Tris·HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$/0.25 mM dithiothreitol/0.2 mM phenylmethanesulfonyl fluoride, centrifuged at 15,000 × *g* for 15 min, passed through a 3-ml column of Bio-Rex 70 (Bio-Rad) to remove most of the basic proteins, and loaded onto 10–30% (vol/vol) glycerol gradients in the same buffer (without phenylmethanesulfonyl fluoride). After centrifugation for 4 hr (for 42S particles) or 20 hr (for 7S particles) at 40,000 rpm in a Beckman SW 41 rotor at 2°C, the particles were located by their absorbance at 260 nm. For DNase protection experiments, 7S particles were subjected to a second round of gradient centrifugation and then passed through a Sephadex G-50 column in 10 mM Tris·HCl, pH 7.5/0.1 mM EDTA. They were then about 80% pure, there being about 10 minor contaminants visible on $NaDodSO_4$ gels.

Purification of the 7S Particle Protein. Gradient fractions containing 15 A_{260} units of 7S particles were adjusted to 5 mM EDTA and loaded onto a 1-ml DEAE-cellulose column (Whatman DE-52, preequilibrated with 10 mM Tris·HCl, pH 7.5). The column was washed with 20 ml of 0.2 M KCl/20 mM Tris·HCl, pH 7.5/0.25 mM dithiothreitol, then with 4 ml of 50 mM Tris·HCl, pH 7.5/0.25 mM dithiothreitol, and the protein was eluted with 7 M urea/50 mM Tris·HCl, pH 7.5/2.5 mM dithiothreitol. Protein-containing fractions were pooled, passed through a 2-ml column of DEAE-Sephadex in the same buffer, dialyzed extensively against 20% (vol/vol) glycerol/50 mM KCl/2 mM $MgCl_2$/2 mM dithiothreitol/20 mM Tris·HCl, pH 7.5, and frozen in 100-μl portions at −70°C.

Labeling and Analysis of Proteins. Protein samples were precipitated with trichloroacetic acid, redissolved in 10 μl of 8 M urea/1 mM dithiothreitol/0.15 M Tris·HCl, pH 8.8, and incubated for 1 hr at 37°C with 5 μl of 16 mM iodo[^{14}C]acetate (Amersham). The mix was diluted 1:2 or more with $NaDodSO_4$ gel sample buffer and electrophoresed on a 10% polyacrylamide gel in the buffer system of Laemmli (12). Protein bands were excised from the stained dried gel and subjected to partial proteolysis as described by Cleveland *et al.* (13).

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Abbreviations: bp, base pair(s); tDNA, tRNA gene.

DNase Protection. DNA fragments labeled at their 5′ termini with ^{32}P were prepared by standard procedures (2, 3). pXbsΔ5′ −15L DNA (2) was digested with restriction endonuclease *Eco*RI, labeled with polynucleotide kinase, and redigested with *Hha* I, and the 389-base-pair (bp) gene-containing fragment was isolated by electrophoresis on a polyacrylamide gel. This fragment was labeled at the 5′ end of the coding strand, 173 bp from the 3′ end of the gene. To label the noncoding strand, pXbs201 or pXbsΔ3′ +124R DNA (3) was digested with *Hin*dIII, labeled with kinase, and redigested with *Alu* I, and the gene-containing fragment (541 or 495 bp, respectively) was isolated. The label was 55 bp from the 5′ end of the gene. Binding reaction mixtures (10 μl) contained 6% glycerol, 7 mM $MgCl_2$, 70 mM NH_4Cl, 10 mM Hepes at pH 7.5, bovine serum albumin at 20 μg/ml, and particles or protein as specified in the figure legends. The mixtures were preincubated for 15 min at 21°C before the DNA fragments (0.04–0.15 pmol) were added. After 10 min, 10 ng of DNase I was added, followed 1 min later by 40 μl of 10 mM Tris·HCl, pH 8.0/20 mM EDTA/0.2% $NaDodSO_4$, and 40 μg of sonicated calf thymus DNA per ml. The DNA fragments were extracted with phenol, precipitated with ethanol, denatured, and electrophoresed on 8% polyacrylamide gels containing 7 M urea (2). *Hpa* II digestion products of pBR322 were used as size markers.

RESULTS

5S RNA Inhibits Its Own Synthesis *in Vitro*. To test the effect of 5S RNA on transcription, plasmids containing one repeating unit of *X. borealis* somatic 5S DNA (pXbs1) or the VA genes of adenovirus (VA DNA) were mixed and transcribed in the presence of increasing amounts of 5S RNA. As a control, the effects of *E. coli* 4S RNA were also tested. Fig. 1 shows that 5S RNA at 5–40 μg/ml inhibited 5S RNA synthesis while actually stimulating synthesis of VA RNA. Transcription of a tRNA gene (tDNA) was also stimulated by 5S RNA in the presence of 5S DNA (see Fig. 4). Because addition of 5S RNA did not enhance VA RNA or tRNA synthesis in the absence of 5S DNA, we assume that this stimulation reflects the relief of competition for common factors involved in transcription. In contrast to 5S RNA, *E. coli* 4S RNA stimulated both 5S RNA and VA RNA synthesis slightly at low concentrations and was somewhat inhibitory at higher concentrations, but showed little specificity in its effects (Fig. 1).

It has been shown previously that a cloned region composed of residues 41–87 of the 5S gene is sufficient to direct accurate initiation of transcription in this cell-free system (3). Because this gene fragment lacks the normal termination site, transcription continues into the plasmid DNA and terminates at

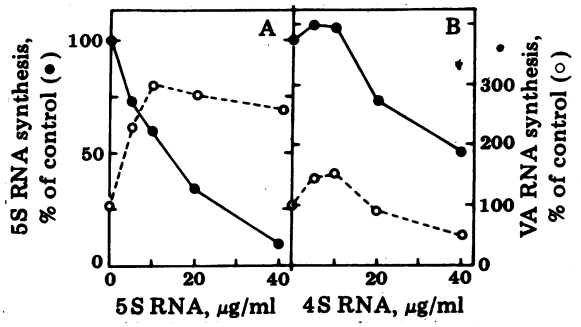

FIG. 1. Effect of 5S RNA on transcription. Incubation mixtures contained pXbs1 and VA DNA each at 5 μg/ml. After 75 min the products were separated on a gel, and the 5S and VA RNA bands were cut out and their radioactivities were measured. The results are expressed as % of cpm in the control (an incubation without added RNA). (*A*) 5S RNA added. (*B*) *E. coli* 4S RNA added. ○, Radioactivity in VA RNA; ●, radioactivity in 5S RNA.

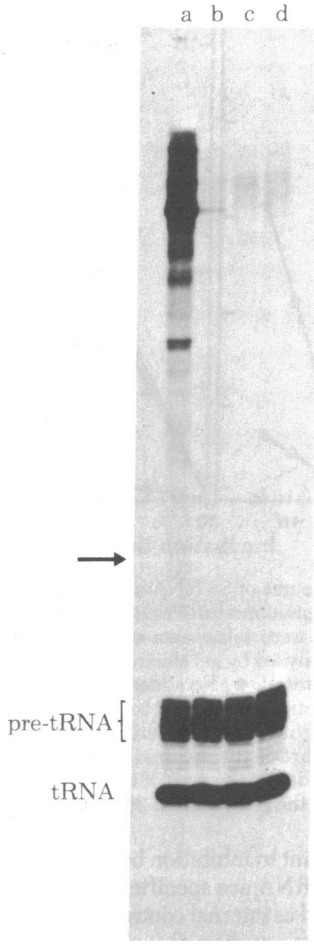

FIG. 2. Effect of 5S RNA on transcription of a cloned internal fragment of the 5S RNA gene. The RNAs obtained after 75 min of transcription were analyzed by gel electrophoresis and autoradiography. The arrow indicates the expected position of 5S RNA. Reaction mixtures contained, per ml: track a, 10 μg of pXbsInt 41/87 R DNA containing residues 41–87 of the 5S gene and 5 μg of tDNA (a cloned *Drosophila* arginine tRNA gene); track b, as for a, plus 20 μg of 5S RNA; track c, 10 μg of pBR322 DNA and 5 μg of tDNA; track d, as for c, plus 20 μg of 5S RNA.

multiple sites. The RNA products are thus heterogenous and larger than 5S RNA. Fig. 2 shows that synthesis of these products is inhibited specifically by 5S RNA at concentrations that do not affect transcription of tRNA genes or plasmid DNA. Thus, 5S RNA appears to interfere with a process that involves the internal control region of the 5S RNA gene.

5S RNA Affects the Formation of an Active Transcription Complex. Fig. 3*A* shows a time course of 5S RNA synthesis directed by pXbs1 DNA in the cell-free system. There is a characteristic lag of 15–20 min before the maximal rate of transcription is achieved. This lag can be eliminated by preincubation of the DNA with the nuclear extract (1), and it presumably reflects organization of the DNA into a transcriptionally active state. When 5S RNA was added at the same time as the DNA, the kinetics of transcription were unaffected, but the overall rate of synthesis was decreased (Fig. 3*A*). By contrast, when 5S RNA was added after preincubation of the extract and DNA for 30 min (Fig. 3*B*), it had no discernible effect for 40 min; thereafter, the transcription rate fell slowly. The amount of RNA synthesized before inhibition was observed in this type of experiment corresponds to at least two molecules per input gene. Thus, 5S RNA inhibits the formation of an active transcription complex, but once formed, such a complex

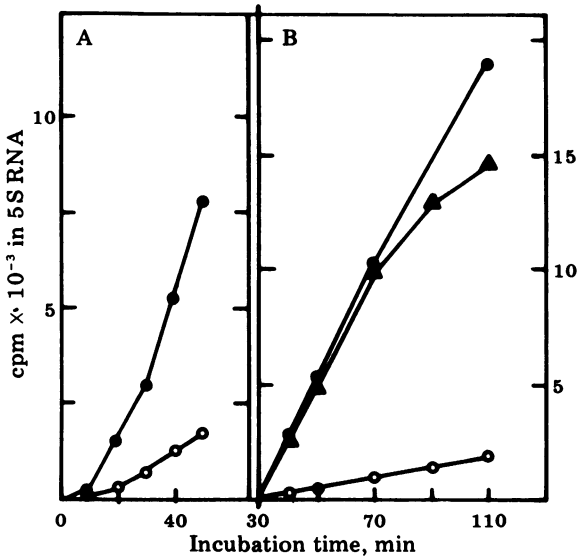

FIG. 3. Time course of 5S RNA inhibition of transcription. (*A*) Aliquots of transcription mixtures containing pXbs1 DNA and tDNA (each at 6.7 μg/ml) were withdrawn at various times and the radioactive RNA was analyzed by gel electrophoresis. Radioactivity in the 5S RNA band is plotted. ●, No added RNA; ○, plus 5S RNA at 27 μg/ml. (*B*) The reaction was similar to *A* except that pXbs1 and VA DNAs (each at 9 μg/ml) were transcribed and [^{32}P]UTP was added 30 min after the start of the incubation. ●, No RNA added; ○, 5S RNA (35 μg/ml) added at the start of the incubation; ▲, 5S RNA (35 μg/ml) added with the [^{32}P]UTP at 30 min.

is relatively resistant to inhibition by added 5S RNA. As before, the effects of 5S RNA are specific; transcription of VA DNA or tDNA, included as internal controls in these experiments, was not inhibited (data not shown).

5S RNA Inhibition Is Competitive with 5S DNA. The simplest explanation of the preceding results would be that 5S RNA binds to a transcription factor and prevents its binding to 5S DNA. If this is true, it should be possible to demonstrate competition between DNA and RNA. Fig. 4*A* shows the effect of varying the DNA concentration on the inhibition by RNA. At concentrations of DNA that are saturating for 5S RNA synthesis (50 μg/ml), addition of 5S RNA at 40 μg/ml had little effect. At lower DNA concentrations, inhibition by RNA was increasingly severe; with DNA at 5 μg/ml or less, marked inhibition was observed with 5S RNA at only 5 μg/ml. However,

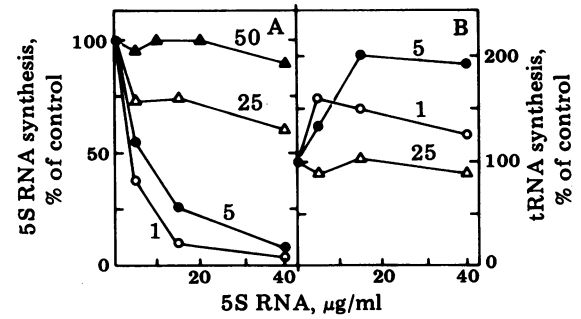

FIG. 4. Effect of DNA concentration on inhibition by 5S RNA. Mixtures of pXbs1 and tDNA were transcribed for 2 hr, and the products were separated on a gel and their radioactivities were measured. In the absence of added RNA approximately 10 times as much 5S RNA was made as tRNA, except at the lowest DNA concentration, where the ratio was approximately 2:1. All values are expressed as % of control (no added RNA). Incubation mixtures contained pXbs1 DNA and tDNA at 1 μg/ml (○), 5 μg/ml (●), or 25 μg/ml (△) each, or pXbs1 DNA alone at 50 μg/ml (▲). (*A*) 5S RNA synthesis; (*B*) tRNA synthesis.

on a molar basis this still represents approximately 100 times as much 5S RNA as 5S RNA genes. Conditions that inhibit 5S RNA synthesis again stimulated transcription of tDNA, which was included as an internal control (Fig. 4*B*).

A Protein That Can Bind Either 5S RNA or the Control Region of 5S DNA. Immature ovaries from *Xenopus* contain large amounts of 5S RNA in the form of ribonucleoprotein particles. About 70% of the 5S RNA is in a 7S complex that contains one molecule each of 5S RNA and a protein whose molecular weight has been estimated to be 45,000 (8). The rest of the 5S RNA is in a 42S particle that also contains tRNA and two proteins, one of which is similar in size to the protein found in 7S particles (8).

Engelke *et al.* (4) have purified a protein from *Xenopus* ovaries that can restore 5S RNA synthesis in a deficient cell extract derived from unfertilized eggs. The requirement for this protein appears to be specific for 5S RNA synthesis; the protein has no effect on the transcription of a *Xenopus* tRNA gene that is known to be transcribed by RNA polymerase III. In addition, this purified transcription factor has been shown to bind to a region within the 5S RNA gene (residues 45–96) that is very similar to the control region defined by deletion analysis [residues 50–80 (2, 3)]. The protein was reported to be about 37,000 molecular weight. Despite the differences in reported molecular weight, it seemed possible to us that the protein complexed with 5S RNA in young oocytes is, in fact, the protein isolated by Engelke *et al.* that binds to the 5S RNA gene and is required for 5S gene transcription. If this were true, it would explain how 5S RNA can inhibit its own synthesis.

To test this hypothesis, 7S and 42S particles were purified from immature ovaries. The proteins isolated from these particles were compared with the purified transcription factor (kindly donated by D. Engelke and R. Roeder) by partial chymotryptic digestion of the proteins labeled with iodo[^{14}C]-acetate (Fig. 5). The 7S particle contains a mixture of two closely related proteins, which are not fully resolved from each other on $NaDodSO_4$ gels. The smaller of these appears to be identical to the transcription factor purified by Engelke *et al.* (4). In several preparations, the ratio of the two protein forms has varied, suggesting that the smaller one may be a degradation product of the larger. We have been unable to eliminate the smaller protein by addition of a protease inhibitor (phenylmethanesulfonyl fluoride) during isolation or by homogenizing ovaries directly in $NaDodSO_4$. We therefore cannot rule out the possibility that both forms exist *in vivo* in the 7S particle.

We have also compared the protein in the 7S particle to the one of similar size in the 42S particle. Although previously thought to be the same (8), these proteins yield different chymotryptic digestion products (Fig. 5). Identical results were obtained when the particles were treated with 0.5 M KCl (which disrupts 42S particles) or with $NaDodSO_4$ sample buffer containing 0.1 M dithiothreitol before trichloroacetic acid precipitation and labeling in 7 M urea.

The intact 7S and 42S particles and the proteins purified from them were tested for their ability to bind 5S DNA. We used the DNase protection technique (14), in which binding of a protein to a specific region of DNA is indicated by protection of that region from DNase digestion. By using a DNA fragment labeled at one end and sizing the partial digestion products on a sequencing gel, the position of the protected region can be determined accurately. The DNA used in the experiment shown in Fig. 6, tracks a–d, was labeled at the 5′ end of the coding strand. When intact 7S particles were mixed with this DNA, no specific binding was detected. However, when the particles were treated with pancreatic ribonuclease before addition to the DNA, the released protein clearly protected residues 45–96

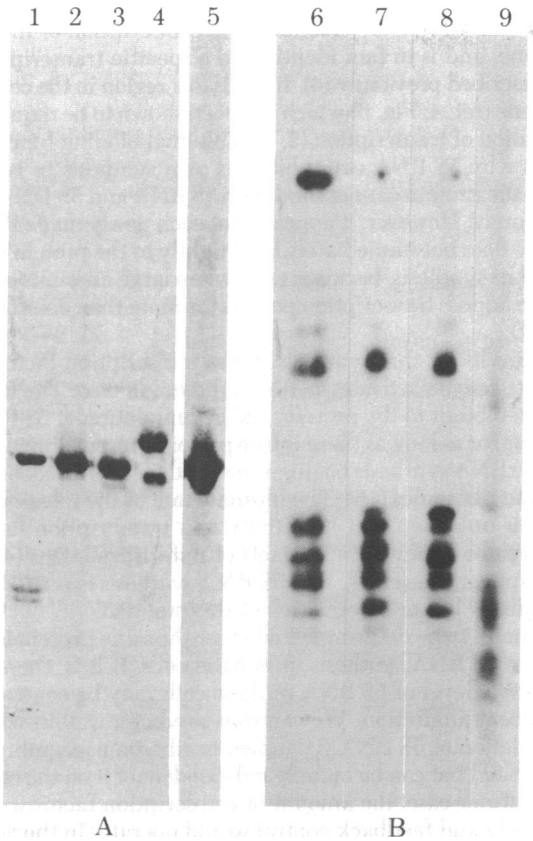

FIG. 5. Analysis of purified proteins. (*A*) Stained $NaDodSO_4$ gel showing: 1, creatine kinase, molecular weight 40,000; 2, purified 7S protein; 3, protein from purified 7S particles; 4, protein from purified 42S particles; 5, a higher loading of purified 7S protein to give an indication of its purity. (*B*) Fluorograph of a $NaDodSO_4$ gel, showing the partial chymotryptic digestion products of carboxy[^{14}C]methylated proteins that were cut out of another $NaDodSO_4$ gel. The protein band from 7S particles was cut in half, and the upper and lower portions were analyzed separately. Track 6, transcription factor isolated by Engelke *et al.* (4); 7, more rapidly migrating portion of the 7S protein; 8, more slowly migrating portion of the 7S protein; 9, smaller protein from 42S particles.

of the 5S RNA gene from nuclease digestion (track c). Ribonuclease alone did not affect the digestion pattern.

Similar results were obtained with DNA labeled at the 5′ end of the noncoding strand (Fig. 6, tracks e and f). Again, 7S particles did not bind to 5S DNA unless treated with ribonuclease; they then protected the same region (residues 45–96) but caused enhanced cleavage at (approximately) residues 92, 72–73, and 59–60. The exact reason for the enhanced cleavages is not known, but similar phenomena have been noted previously in this kind of experiment (14). In contrast to 7S particles, 42S particles did not protect any specific region of the DNA, either with or without ribonuclease treatment (tracks g and h). In control experiments neither ribonuclease-treated 7S particles nor 42S particles protected tDNA from DNase (data not shown).

We next purified the protein from the 7S particles, free of RNA. To do this, we made use of the observation that the particle, but not the protein, binds tightly to DEAE-cellulose. Crude 7S particles were loaded onto a DEAE-cellulose column, the column was washed with 0.2 M KCl, and the protein was eluted with 7 M urea at low salt, leaving the RNA bound to the column. Protein prepared in this was 95–98% pure as judged by $NaDodSO_4$ gel electrophoresis (Fig. 5) and, after dialysis to remove the urea, it had DNA-binding activity. Track j of Fig. 6 shows that the purified protein gave a DNase protection pattern identical to the one obtained with ribonuclease-treated 7S particles.

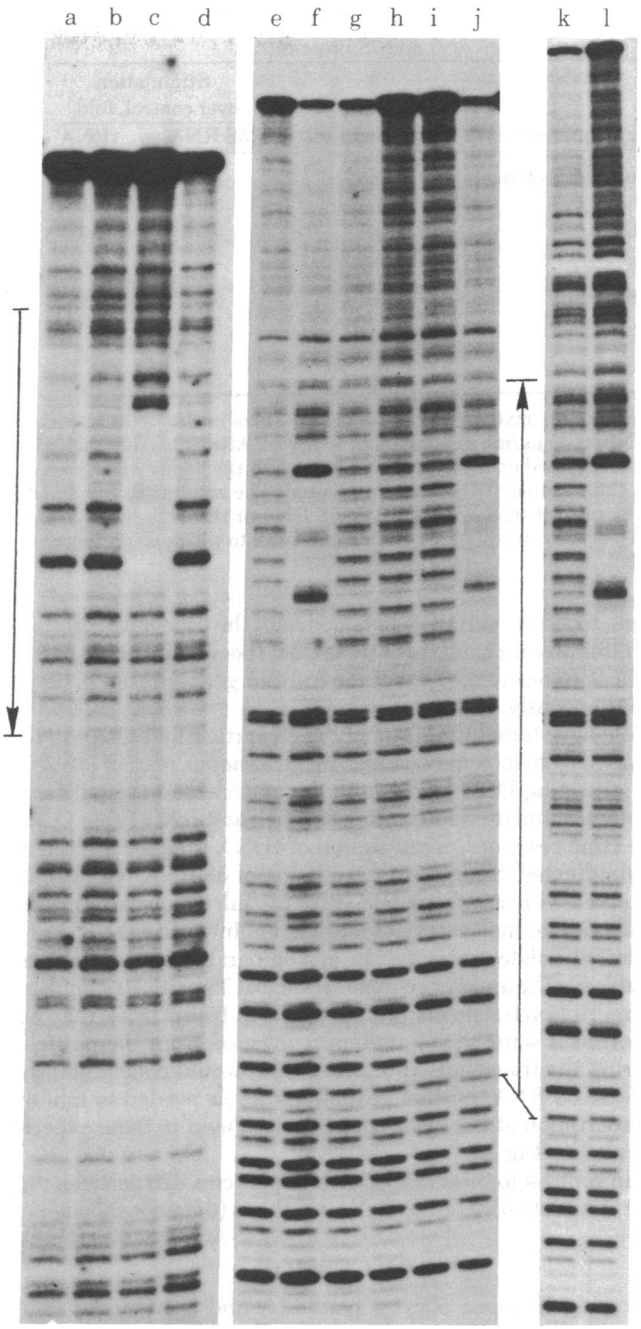

FIG. 6. Protection of 5S DNA from DNase. Preparation of DNA fragments, binding, DNase digestion, and electrophoresis of the DNA are described in *Materials and Methods*. DNA fragments were labeled at the 5′ end of the coding (a–d) or noncoding (e–l) strand. Arrows delimit the borders of the 5S RNA gene, and indicate the direction of transcription. The protected regions on both strands correspond approximately to residues 45–96 of the gene. Binding reaction mixtures contained the following additions: a, none; b, 7S particles (100 ng of protein); c, 7S particles plus RNase (10 ng); d, RNase; e, 7S particles; f, 7S particles plus RNase; g, 42S particles (260 ng of protein); h, 42S particles plus RNase; i, none; j, purified 7S protein (50 ng); k, purified 7S protein plus 5S RNA (180 ng); l, purified 7S protein plus *E. coli* 4S RNA (180 ng).

Preincubation of the purified protein with 5S RNA abolished its DNA-binding activity, whereas preincubation with the same amount of *E. coli* 4S RNA had no effect (tracks k and l). This

Table 1. Stimulation of 5S RNA synthesis by 7S particle and 7S protein

Factor added	Conc., μg protein/ml	Stimulation over control, fold 5S RNA	tRNA
Purified protein	0.45	1.6	1.0
	1.35	3.8	0.9
	4.5	3.5	0.7
	13.5	2.9	0.5
7S particle	0.75	2.5	1.2
	2.25	5.9	1.1
	7.5	2.2	0.5
	22.5	5.3	0.7

Incubation mixtures (20 μl) contained 5 μl of nuclear extract, pXbs 1 DNA at 25 μg/ml, and tDNA at 40 μg/ml. After 1 hr, the products were separated on a polyacrylamide gel, and the bands were excised and their radioactivities were measured. The results are expressed as the ratio of the radioactivity in 5S RNA or tRNA to the radioactivity in the corresponding RNA in a control to which no protein was added.

result, together with those obtained with the particles, indicates that this protein has the ability to bind specifically either to 5S RNA or to residues 45–96 of the 5S RNA gene, but not to both simultaneously.

We have tested the effect of the 7S particle and the protein purified from it on transcription in the *Xenopus* nuclear extract. At saturating DNA levels, both the particle and the purified protein stimulate transcription as much as 6-fold (Table 1). In the same reactions, transcription of tDNA is either inhibited or unaffected. Significant stimulation of 5S RNA synthesis is observed with as little protein as 0.5 μg/ml, which corresponds to about two molecules per input gene. Interestingly, 7S particles stimulate transcription at comparable concentrations (Table 1). The apparent contradiction that 7S particles stimulate transcription but do not protect the DNA from DNase may be resolved if some active protein is released from the particle during the transcription reaction. This would concomitantly release 5S RNA, but not at the high levels needed to inhibit transcription at the DNA concentration used in these experiments (see Fig. 4).

In contrast to these results with 7S particles, 42S particles did not stimulate 5S RNA or tRNA synthesis (data not shown).

DISCUSSION

Growing *Xenopus* oocytes synthesize and accumulate ribosomes at least 1000 times more rapidly than the most active somatic cells. These ribosomes are used subsequently for early embryogenesis. Augmented synthesis of 18S and 28S rRNAs is made possible by amplification of their structural genes (reviewed in ref. 15). The 5S RNA genes are not amplified, but thousands of genomic copies of the genes, which are not expressed in somatic cells, are transcribed in the oocyte (15). Although there are about 100,000 5S RNA genes in an oocyte of *X. laevis*, this is still 20-fold fewer than the number of amplified 18S and 28S rRNA genes. Perhaps because of this numerical imbalance, there exists in young oocytes a mechanism that allows the synthesis and accumulation of massive amounts of 5S RNA and tRNA during the 1- to 2-month period before 18S and 28S rRNA synthesis begins (5–7). Most of the accumulated 5S RNA is complexed with a protein in the form of a 7S cytoplasmic particle (8). This particle becomes extremely abundant: the protein in it can constitute up to 15% of the soluble protein in immature oocytes.

We have shown that the protein in the 7S particles can function as a stimulatory factor in the transcription of the 5S RNA gene, and is in fact identical to a specific transcription factor described previously (4). It binds to a region in the center of the gene (ref. 4; Fig. 6) which has been shown to be required for initiation of transcription (2, 3). This dual binding function explains why 5S RNA can inhibit its own synthesis *in vitro*, because the protein cannot bind both 5S RNA and 5S DNA simultaneously. However, it appears that each newly made RNA molecule does not immediately bind tightly to the protein that directed its synthesis, because, even when large amounts of 5S RNA are added, transcription persists for more than one round (Fig. 3*B*).

It seems likely that synthesis of the transcription factor in immature oocytes activates 5S RNA synthesis *in vivo*. The RNA made then binds to the protein, and accumulation of 5S RNA can occur for as long as there is free protein present. Once 18S and 28S rRNA synthesis begins, the stored 5S RNA is incorporated into ribosomes (16). The ultimate fate of the released 7S protein is unknown, but its activity as a transcription factor appears to be absent from extracts of unfertilized eggs (4). It would appear, therefore, that 5S RNA synthesis is turned off at meiosis by loss or inactivation of this protein.

It remains to be determined whether the same protein is involved in 5S RNA synthesis in somatic cells. If it is, then the steady-state level of 5S RNA in these cells may be controlled by feedback inhibition. We can thus predict a way in which accumulation of 5S RNA is coupled to ribosome assembly on the one hand but can be entirely independent of it on the other. In the former case, the amount of transcription factor would be limiting and feedback control would operate. In the latter case, this control would be overridden by massive synthesis of transcription factor, allowing accumulation of 5S RNA in the absence of ribosome synthesis, as occurs in immature oocytes.

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