Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7498–7502, November 1987
Biochemistry

Mouse and frog violate the paradigm of species-specific transcription of ribosomal RNA genes

(rDNA/species specificity/RNA polymerase I)

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Communicated by Oscar L. Miller, July 20, 1987 (received for review April 24, 1987)

ABSTRACT **Transcription of ribosomal RNA genes by RNA polymerase I is generally accepted as being highly species specific, a conclusion based on numerous reports that rRNA genes of one species are not transcribed by factors of even closely related species. It thus was striking to find that cloned rDNA from the frog *Xenopus laevis* is specifically transcribed in extracts prepared from mouse cells. The data in this paper demonstrate that this heterologous transcription is due to a normal initiation process and not to a fortuitous event. Transcription of *Xenopus* rDNA in the mouse cell extract is directed by the same large promoter (residue −141 to +6) that is utilized to promote the synthesis of frog rRNA in homologous *Xenopus* systems. Moreover, the same factors of the mouse cell extract that transcribe the homologous mouse rDNA also catalyze transcription from the *X. laevis* rDNA promoter. We conclude that polymerase I transcriptional machinery does not evolve as rapidly as prior studies would suggest.**

In the past 6 years, great progress has been made toward understanding the specific transcription catalyzed by each of the three classes of eukaryotic RNA polymerase. Accurate *in vitro* and *in vivo* transcription systems have been developed, transcriptional initiation sites have been identified, and the nucleotide sequences involved in promoting synthesis have been located. Transcription catalyzed by RNA polymerases II and III has been found to exhibit only a rather limited degree of species specificity. Accordingly, regions of RNA polymerase II and III promoters tend to be conserved quite well between different species, the classic example being the "TATAA" box, which was noted as a sequence motif that is present in most mRNA-encoding genes prior to its identification as a polymerase II promoter element (1).

For the rRNA genes transcribed by polymerase I, however, very little sequence homology has been detected between the rDNA promoter regions of various eukaryotic species (2, 3). Even within mammals, the major sequence conservation extends only from residues +2 to +18 (4), a region that is largely downstream from the minimal essential promoter region (approximately from residue −35 to +5; reviewed in ref. 3). Since the polymerase I transcription machinery evidently recognizes only one kind of gene, and rDNA spacer sequences evolve rapidly, it was not surprising to find that rDNA transcription exhibits a significant degree of species selectivity. For example, mouse and human rRNA genes are not transcribed in extracts of the other species, even though these extracts transcribe the homologous species rRNA genes very actively (5–8). Moreover, *Acanthamoeba*, *Xenopus*, and human rRNA genes are evidently not transcribed in extracts from any of the other three species (5) and the polymerase I transcription machinery of *Drosophila melanogaster* does not appear to be active on *Drosophila virilis* rRNA genes (9). In general, successful cross-species transcription by polymerase I has only been reported for closely related species. Examples of this include the transcription of *Xenopus laevis* genes in *Xenopus borealis* oocytes (10), monkey genes in human extracts (8), rat genes in mouse extracts (6), and mouse genes in transfected rat and hamster cells (11–13).

However, two instances have been reported in which rRNA genes are evidently transcribed by factors from a species that is not closely related. First, when rDNA isolated from the water beetle *Dytiscus* is injected into *Xenopus* oocytes and the resultant chromatin is examined by electron microscopy, many molecules are seen to have assembled transcription units indistinguishable in length and packing density from those found in *Dytiscus* nucleoli *in vivo* (14). This result demonstrates that *Xenopus* transcription components are active on *Dytiscus* rRNA genes, although it remains to be shown that the synthesis is due to accurate initiation by RNA polymerase I.

The second instance in which rRNA genes are reported to be transcribed by components of a distantly related species occurs when cloned *Xenopus* rDNA reacts in an S-100 extract derived from mouse tissue culture cells (15). In this case, transcription was shown to initiate specifically in the region of the natural frog rRNA initiation site and to utilize RNA polymerase I. However, from this study it remained unclear which sequences of the frog rDNA directed the heterologous transcription. Since nucleotides −10 to +1 of *X. laevis* rDNA show a striking degree of similarity with the analogous region of mouse rDNA (15) and this segment substantially coincides with the minimal region that can direct transcription in homologous *Xenopus* systems (residues −7 to approximately +4) (16), it seemed possible that this short sequence was responsible for the transcription of frog rDNA in the mouse extract. Alternatively, the heterologous transcription might require the entire ≈150-base-pair frog rDNA promoter region that is needed to obtain maximal levels of transcription under most conditions in the homologous frog systems (16, 17).

The experiments presented in this paper further examine the heterologous transcription of frog rDNA in the mouse cell extract, focusing both on the cis-acting control elements and on the requisite soluble factors. We conclude that the promotion of this heterologous transcription is not fortuitous, for the complete ≈150-base-pair *Xenopus* rDNA promoter that is recognized in homologous frog systems is also required for frog rRNA synthesis in the mouse extract. Furthermore, this large promoter is evidently recognized by the same limiting trans-acting mouse factors that catalyze transcription of the mouse rRNA gene. These results suggest that there is a significantly greater degree of conservation in the process of rDNA transcriptional initiation than had previously been assumed.

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MATERIALS AND METHODS

Transcription Analysis. S-100 extracts were prepared from logarithmically dividing mouse L1210 tissue culture cells according to the methods of Miller and Sollner-Webb (18). S-100 transcription reactions of 25 μl contained 7 μl of extract and were brought to 15 mM Hepes, pH 7.9/10% (vol/vol) glycerol/0.1 mM EDTA/1 mM dithiothreitol/85 mM KCl/3.5 mM $MgCl_2$. Reactions also contained 300 μg of α-amanitin per ml, 7–10 μg of the specified cloned *X. laevis* rDNA template per ml, and ribonucleotide triphosphates (rXTPs). To prepare unlabeled RNA, ATP, GTP, UTP, and CTP (500 μM each) were used, whereas to synthesize radioactive transcripts, the CTP concentration was lowered to 10–50 μM and 3 μCi of [α-^{32}P]CTP was added. Reactions were then incubated at 30°C for 1 hr and were terminated by the addition of 180 μl of a solution containing 0.15 M NaCl, 5 mM EDTA, 50 mM Tris·HCl (pH 7.6), 0.3 M NaOAc, 0.5% $NaDodSO_4$/tRNA (50 μg/ml). RNA was isolated by phenol extraction and ethanol precipitation.

For assays involving a preincubation, the initial transcription mixture contained 7 μg of the "first DNA" per ml but lacked rXTPs. After 5 min at 30°C, the reactions were supplemented with 7 μg of the tester frog rDNA template per ml, and a 1-hr synthesis period was initiated by addition of rXTPs and [α-^{32}P]CTP.

Transcription reactions involving mouse factors C and D were as described above, except that the S-100 extract was replaced by 5–10 μl of each factor [kindly provided by John Tower in our laboratory] and 20 units of RNasin (Promega Biotec, Madison, WI) was added. The C and D factors were isolated by DNA cellulose chromatography of the mouse S-100 extract (19). Factor C was then further purified by sequential gradient elutions from DEAE-Sephadex and heparin-Sepharose and then by sucrose gradient centrifugation, yielding an essentially pure preparation of the RNA polymerase I polypeptides (19). Factor D was further purified by gradient elution from heparin-agarose, resulting in ≈5000-fold purification (20).

The RNA products of reactions containing unlabeled rXTPs were analyzed by S1 nuclease mapping or primer extension as described (10, 15). Transcripts synthesized in the presence of radioactive nucleotides were resolved by electrophoresis on 4% acrylamide/7 M urea gels and were visualized by autoradiography (18).

rDNA Templates. The *X. laevis* rDNA plasmid (pXlr315; ref. 16) contains residues −1150 to +115 of the initiation region joined onto 360 base pairs encoding the 3′ end of 28S rRNA region and is cloned between the *Bam*HI and *Eco*RI sites of pBR322. The 5′ deletions were derived from pXlr315 and lack all rDNA residues upstream of the indicated position (16). Transcription of pXlr315 or the 5′ deletions linearized with *Eco*RI produces an ≈470 nucleotide run-off transcript. Two sets of 3′ deletions (16), which lack all rDNA residues downstream of the indicated position, were used. The deletion end point of mutants 3′Δ+13, +6, −9, and −11 is cloned into the *Bam*HI site of pBR322. When linearized with *Eco*RI or with *Hin*cII, 3′Δ+13 yields ≈390 or ≈745 nucleotide run-off transcripts, respectively: 3′Δ+6, 3′Δ−9, and 3′Δ−11 would be expected to yield transcripts that are 7, 22, and 24 nucleotides shorter. The deletion end point of 3′Δ−19 and 3′Δ−52 is cloned into the *Hin*dIII site of pBR322. A transcript of 3′Δ−19 that started at the position corresponding to residue +1 would be ≈470 nucleotides long when the template is truncated with *Hin*cII; a corresponding RNA from 3′Δ−52 would be ≈437 nucleotides long. The mouse rDNA template p5′Sal-Pvu contains mouse rDNA residues −170 to +300 (18).

RESULTS

Earlier experiments had demonstrated that a closed circular plasmid containing the initiation region of the *X. laevis* rRNA gene could direct specific transcription by RNA polymerase I when reacted in an S-100 extract of mouse tissue culture cells (15). As shown in Fig. 1*A*, the cloned frog rRNA gene is also active as a linearized template. The resultant transcript (lane 2), like that catalyzed on closed circular molecules (lane 1), can be detected by S1 nuclease analysis. Transcripts of linearized templates can also be assessed directly by a run-off transcription assay using radioactive rXTPs and templates truncated a few hundred base pairs beyond the initiation site. This is demonstrated in Fig. 1*B*, where ≈315, 435, and 485 nucleotide run-off transcripts are generated from templates truncated these distances downstream of the initiation site. Relative to the homologous *Xenopus in vitro* transcription system, this heterologous transcription is quite active, yielding 10- to 20-fold more transcript molecules per input rDNA molecule (15).

The Frog rDNA Sequences That Direct Transcription in the Mouse S-100 Extract. Immediately surrounding the initiation site, mouse and *Xenopus* rDNA exhibit an 8 of 11 nucleotide sequence identity (residues −10 to +1; ref. 15), which largely overlaps the minimal frog rDNA promoter (16). This suggested that the heterologous rDNA transcription might merely be due to this short (possibly fortuitous) region of sequence homology. Alternatively, if the entire frog rDNA promoter extending out to approximately residue −141 was involved in initiating the heterologous transcription, this would indicate that the recognition of polymerase I promoter sequences by rDNA transcription factors is functionally conserved between mouse and frog. To address this issue, we determined the extent of the frog rDNA sequences that are required to promote initiation in the mouse S-100 extract by assessing the transcriptional capacity of a systematic series of deletion mutants that approach the *X. laevis* rRNA initiation site from the 5′ direction (5′ deletions) or from the 3′ direction (3′

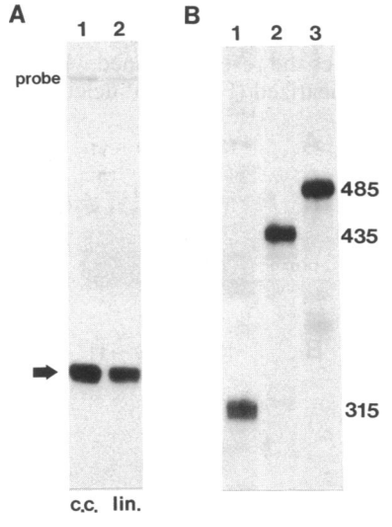

FIG. 1. Transcription of frog rDNA in the mouse S-100 extract. (*A*) *X. laevis* rDNA (plasmid pXlr315) was transcribed as a closed circular plasmid (lane 1) or after linearization at residue +315 (lane 2) in reactions containing unlabeled rXTPs. Resultant RNA was assessed by S1 nuclease analysis. The amount of transcript obtained from closed circular and linear templates is similar, varying by a factor of ≈2 in either direction depending on the extract. (*B*) *X. laevis* rDNA was linearized at residue +315 (lane 1), +435 (lane 2), or +485 (lane 3) and was transcribed in reactions containing radiolabeled rXTPs. Resultant RNA was resolved by electrophoresis and visualized by autoradiography.

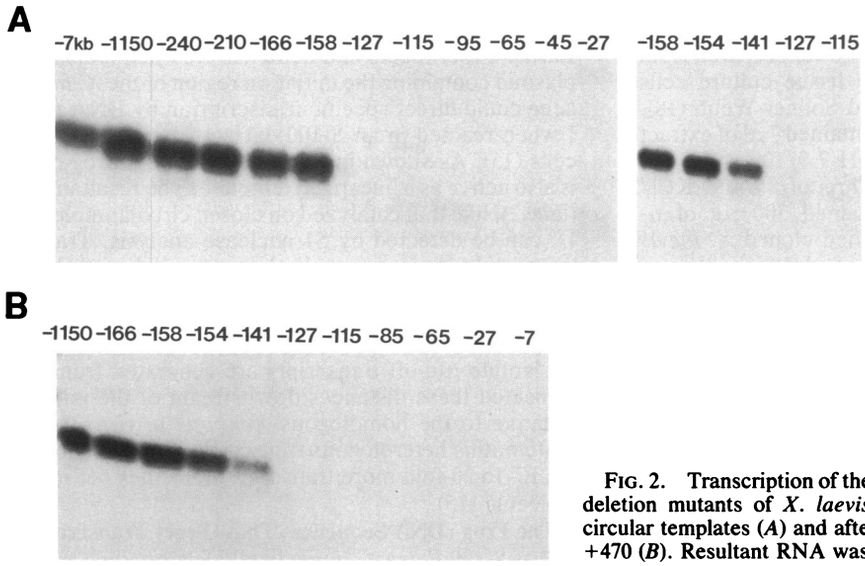

FIG. 2. Transcription of the 5′ deletion mutants. The indicated 5′ deletion mutants of *X. laevis* rDNA were transcribed as closed circular templates (*A*) and after linearization with *Eco*RI at residue +470 (*B*). Resultant RNA was assessed by S1 nuclease analysis.

deletions). Since previous studies of mouse rDNA transcribed in the mouse S-100 extract demonstrated that different promoter domains can be detected with closed circular versus linear template molecules (21, 22), both of these forms of the frog rDNA templates were examined.

Transcription of the frog rDNA 5′ deletion mutants in the mouse S-100 extract is shown in Fig. 2. Both with closed circular plasmids (Fig. 2*A*) and linear molecules (Fig. 2*B*), 5′Δ−154 and all less deleted templates direct transcription at high levels, 5′Δ−141 directs an intermediate level of transcription, and 5′Δ−127 and all more extensively deleted templates are virtually inactive. Precisely the same relative template efficiencies were observed when these 5′ deletion mutants were transcribed using the homologous frog *in vitro* (16) and *in vivo* systems (17). Thus, the promoter sequences that extend in the 5′ direction to approximately residue −141 direct transcriptional initiation of *Xenopus* rDNA in both the heterologous and homologous synthesis systems.

Fig. 3 illustrates the results obtained when closed circular (Fig. 3*A*) and linearized (Fig. 3*B*) 3′ deletion mutants of the

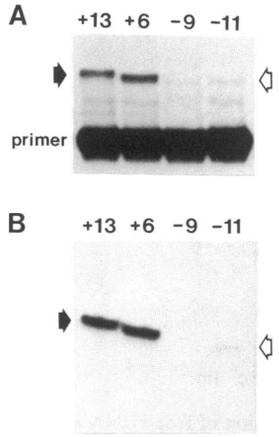

FIG. 3. Transcription of 3′ deletion mutants. (*A*) The indicated 3′ deletion mutants of *X. laevis* rDNA were transcribed as closed circular templates and the reaction products were assessed by primer extension analysis. (*B*) The indicated 3′ deletions were linearized with *Hin*cII, ≈745 nucleotides beyond the initiation site. Transcriptions were conducted in the presence of radiolabeled rXTPs, and resultant RNA was assessed by run-off transcription. Fully consistent results were obtained when the templates were instead truncated with *Pvu* I, ≈915 nucleotides beyond the initiation site.

frog rRNA gene are transcribed in the mouse S-100 extract. The analysis in *A* is by primer extension analysis, while that in *B* is by run-off transcription. Again reproducing the results obtained in the homologous *Xenopus in vitro* and *in vivo* systems (16, 17), efficient transcription is obtained with 3′ deletions that extend into residue +6 but not with 3′Δ−9 or more extensive deletions. Thus, the same large rDNA promoter region, extending from approximately residue −141 to +6, is involved in initiating rRNA synthesis in the heterologous as in the homologous *Xenopus* transcription system.

The Frog rDNA Promoter Contains Two Separate Domains, Each Capable of Directing Transcription. The experiments of Fig. 3 serve to map the major 3′ border of the *Xenopus* rDNA promoter. However, more careful inspection of the run-off transcription data (Fig. 3*B*), as well as of the primer extension data both from the heterologous transcription system (Fig. 3*A*) and from the homologous *Xenopus* oocyte injection system (16), suggests that there may be a small amount of rRNA transcribed from 3′Δ−11. (The positions of these transcripts are indicated by open arrows in Fig. 3.) Since the transcription of radiolabeled run-off RNA is considerably more sensitive for detecting low levels of synthesis, this assay was used to further examine RNA produced from extensive 3′ deletion mutants. In the experiment of Fig. 4, 3′Δ−11 actually initiates transcription at ≈20% the level of the genes bearing an intact promoter (3′Δ+13 and 3′Δ+6). Moreover, the 3′Δ−19 deletion mutant also directs a significant amount of rRNA synthesis, ≈10% of the level of the parental rDNA. [The amount of transcript obtained from 3′Δ−11 and 3′Δ−19 varies somewhat with different extracts (e.g., compare 3′Δ−11 in Figs. 3*B* and 4).] In fact, 3′Δ−52 appears to direct

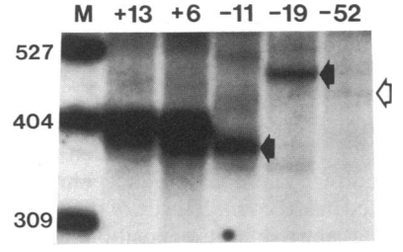

FIG. 4. Transcription of the extensive 3′ deletion mutants. The indicated 3′ deletion mutants of the *X. laevis* rRNA gene were truncated with *Hin*cII and transcribed in reactions containing radioactive rXTPs. Arrows indicate the position predicted for the run-off transcripts from 3′Δ−11, −19, and −52 (see *Materials and Methods*).

a detectable level of specific transcription (indicated by open arrow in Fig. 4). For the 3′Δ−11, −19, and −52 templates, the transcription initiates within the vector sequences that abut the site of the deletion. It should be noted that the rDNA of mutants 3′Δ−19 and −52 is cloned into pBR322 at a different site than is 3′Δ+16, −6, −9, and −11, and hence different length run-off transcripts are expected from these templates. Moreover, since neither of the abutting vector regions bear detectable homology to the rDNA initiation sequence, transcription from these mutants cannot be attributed to fortuitous promoter-like sequences in the vector DNA. These data therefore indicate that sequences of the *X. laevis* rDNA promoter upstream of residue −19 are able to direct specific transcription by RNA polymerase I. This result is intriguing because it is known that *Xenopus* rDNA transcription can also be directed by the proximal promoter region (residues −7 to +4) in the absence of upstream rDNA sequences (16). Thus, both upstream and downstream domains of the rDNA promoter can independently initiate transcription under appropriate conditions.

Mouse Transcription Factors Required for Synthesis of Frog rRNA. We next asked whether the factors of the mouse S-100 extract that catalyze synthesis of mouse rRNA were also responsible for initiating transcription of the frog genes. Prior studies have revealed that two chromatographic fractions of the mouse S-100 cell extract are sufficient to transcribe mouse rDNA (20). Component D binds stably to sequences of the mouse rDNA promoter and has been purified ≈5000-fold. Factor C is evidently an activated subform of RNA polymerase I; it binds to the factor D–rDNA complex and has been purified to yield a virtually homogeneous preparation of the polymerase I polypeptides (19).

To determine whether the mouse rDNA transcription factors are involved in catalyzing the heterologous transcription of frog rRNA genes, we tested the ability of a mouse rDNA template to compete with *X. laevis* rDNA for soluble factors. The S-100 extract was preincubated with p5′Sal-Pvu, a plasmid bearing a cloned mouse rDNA template to which factors D and C stably bind (20, 23). As a control, the extract was also preincubated with pBR322 to which the rDNA transcription factors do not stably bind (20, 23). The ability of these pretreated extracts to transcribe a subsequently added *X. laevis* rRNA gene is displayed in Fig. 5. Preincubation with pBR322 DNA (lane 2) did not impair transcription of the subsequently added frog gene, for the amount of run-off transcript equalled that obtained from reactions preincubated in the absence of DNA (lane 1). In contrast,

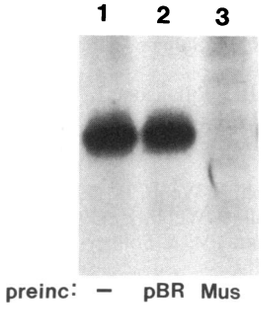

FIG. 5. Competition between mouse and frog rRNA genes for factors of the mouse S-100 extract. Reactions containing the mouse S-100 extract and lacking rXTPs were preincubated for 5 min in the absence of DNA (lane 1), in the presence of closed circular pBR322 (lane 2), or in the presence of the closed circular mouse rDNA-containing plasmid, p5′Sal-Pvu (lane 3). The frog template pXlr315, truncated with *Eco*RI, was then added and a 45-min synthesis period was initiated by addition of radiolabeled rXTPs. The region of the autoradiogram containing the 470-nucleotide frog run-off transcript is shown.

prebinding of the S-100 factors to mouse rDNA abolished transcription of the subsequently added frog template (lane 3). Thus, transcription of mouse and frog rDNA in the mouse S-100 extract involves at least one common limiting factor that stably binds to the rDNA promoter.

Like the homologous transcription of mouse rDNA, the heterologous transcription of frog rDNA involves both the C and D components of the mouse cell extract. This is shown in Fig. 6, where frog rDNA is transcribed by the combination of isolated mouse factors C and D. As reported earlier for the transcription of mouse rDNA (20), the amount of frog rRNA synthesized in these reconstituted reactions is proportional to the amounts of both factor C and factor D (data not shown). Fig. 6 also demonstrates that transcription by these combined factors, like that in whole S-100 extracts, is responsive to the upstream domain of the *Xenopus* rDNA promoter. The template 5′Δ−141 and all less extensive 5′ deletions are actively transcribed, while 5′Δ−127 and the more extensive deletions are not. The combined data of Figs. 5 and 6 thus indicate that the same mouse factors C and D that catalyze the homologous transcription are also required to direct the heterologous transcription of frog rDNA.

DISCUSSION

The species-selective nature of eukaryotic rRNA synthesis has been examined in numerous RNA polymerase I transcription systems. While the vast majority of the data supports the notion that transcription of rRNA genes only functions across very closely related species (5–8), two instances appear to contradict this view. First, purified cellular rDNA of the water beetle *Dytiscus* that had been injected into *Xenopus* oocytes was observed to direct formation of apparently normal transcription units when analyzed by electron microscopy using the Miller spreading technique (14). The second reported instance of heterologous rDNA transcription is more amenable to biochemical analysis. This is the specific transcription of cloned *Xenopus* rDNA in an S-100 extract derived from mouse cells (15). However, despite the fact that this heterologous transcription is more efficient than is the homologous *in vitro* transcription of frog rDNA (15), it was not clear whether the interactions involved in its promotion actually mimicked those in the homologous system. To address this question, we have analyzed both the template sequences and the trans-acting factors responsible for synthesis of frog rRNA in the mouse S-100 extract.

The cis-acting elements that direct the heterologous transcription were assessed by measuring the synthetic capacity of series of 5′ and 3′ deletions of the frog rDNA promoter region. Earlier studies involving homologous *in vitro* and *in vivo* frog rDNA transcription systems had demonstrated that the *X. laevis* rDNA promoter consists of a "proximal"

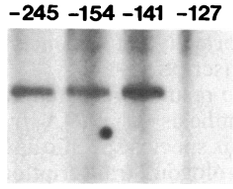

FIG. 6. Transcription of a *X. laevis* rRNA gene by mouse polymerase I factors C and D. Reaction mixtures containing the isolated mouse transcription factors C and D were incubated in the presence of radioactive rXTPs and the indicated 5′ deletion mutants that had been truncated with *Eco*RI at position +470. The same result was obtained whether the reaction utilized partially purified factors C and D (see above) or extensively purified transcription factors (data not shown).

domain (approximately from residues −7 to +6) and upstream stimulatory sequences that extend in the 5′ direction to approximately residue −141 (16, 17). The data of Figs. 2 and 3 show that this entire ≈150-base-pair promoter also directs the heterologous synthesis of frog rRNA in the mouse S-100 extract. These results demonstrate that mammalian factors can efficiently recognize and initiate transcription from the rDNA promoter of a species as distant as an amphibian.

This heterologous transcription not only utilizes the natural frog rDNA promoter region but also involves the same polymerase I factors that catalyze transcription of the homologous mouse rDNA (Figs. 5 and 6). These essential mouse transcription components include factor D, which stably binds to the mouse rDNA promoter region (20), and factor C, which is a subform of RNA polymerase I that is activated for specific rDNA initiation (19) and can stably bind to the factor D/rDNA promoter complex (20). Prebinding competition studies demonstrate that mouse promoter-binding factors are also essential for the heterologous synthesis of frog rRNA (Fig. 5). Furthermore, the isolated mouse factors C and D in combination initiate transcription on the frog rRNA gene, and this requires the same promoter region extending upstream to approximately residue −141 that is utilized in unfractionated S-100 extracts. These observations all imply that the same mouse transcription factors that are needed to transcribe the mouse rRNA gene are sufficient and necessary to catalyze the heterologous synthesis of frog rRNA.

Since the mouse factors transcribe frog as well as mouse rRNA genes, one might anticipate a significant degree of sequence homology between the rDNA promoter elements of frog and mouse. As noted above, there is an 8 of 11 nucleotide sequence identity between these two species rDNA initiation sites (residues −10 and +1), which may facilitate recognition of the frog proximal promoter region by the mouse transcription factors. In addition, segments that contain the upstream portion of the two species' rDNA promoters exhibit a 13 of 17 nucleotide sequence identity (*Xenopus* rDNA residues −138 to −122 and mouse rDNA residues −153 to −137). It remains to be verified, however, that these homologous sequences indeed serve an analogous function in directing rDNA transcription.

Another conclusion obtained from our studies is that more than one domain of the *Xenopus* rDNA promoter can direct transcriptional initiation. The 3′Δ−11 and 3′Δ−19 *Xenopus* deletion mutants were found to be transcribed at a significant level, and even 3′Δ−52 appears to yield a small amount of transcript (Fig. 4). This result is striking in light of earlier studies, which showed that the proximal domain of the *Xenopus* promoter (residue −7 to +4) is also capable of directing transcription both *in vitro* and *in vivo* (16). From these data we conclude that the proximal and the upstream promoter domains are able to independently bind essential polymerase I transcription factors and initiate specific rRNA synthesis. We speculate that these two domains, rather than being redundant, normally act in concert to direct maximal levels of rDNA transcription.

In conclusion, the results presented in this paper demonstrate that the synthesis of rRNA by eukaryotic RNA polymerase I is not as species specific as has been previously assumed. The heterologous transcription of frog rDNA by factors of mouse cells is shown to reproduce the natural initiation process, since it utilizes the same 150-base-pair promoter region and the same trans-acting factors as do the respective homologous transcription systems. Although a number of other cross-species rRNA synthesis reactions have been reported not to occur (5–8), it is possible that alternative reaction conditions might allow demonstration of such heterologous transcriptions to proceed. In this regard, frog rDNA was originally thought to not be transcribed by factors of the mouse extract (5), but it was subsequently demonstrated (15) that this heterologous transcription does occur and that the transcriptional efficiency increases to ≈20% that obtained with the homologous mouse genes when the magnesium concentration is lowered from 5 to 3.5 mM. Clearly, the fact that mammalian factors specifically initiate transcription on the rRNA genes of a species as distant as an amphibian is surprising. This result suggests that interactions involved in promoting rDNA transcription may not evolve at the high rate that has frequently been assumed to date.

The deletion mutants used in this paper were prepared in an earlier collaborative study with Ron Reeder's laboratory (16) and we thank him for their subsequent use. We also gratefully acknowledge John Tower for providing the purified mouse transcription factors. For patient help in preparing this manuscript, we also thank Ms. Sue Millionie. This work was supported by National Institutes of Health Grant GM27720.

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