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Accurate transcription of cloned *Xenopus* rRNA genes by RNA polymerase I: demonstration by S1 nuclease mapping

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ABSTRACT

We have demonstrated faithful transcriptional initiation of cloned Xenopus rRNA genes upon injection into Xenopus oocytes. This observation has been made possible by the use of an S1 nuclease assay that is both sensitive and quantitative. In order to detect rRNA synthesis from the injected template above the large background of rRNA endogenously present in oocytes, the divergence of ribosomal DNA sequences between two Xenopus species was utilized. Cloned X. laevis ribosomal DNA was injected into the nuclei of X. borealis oocytes. Total oocyte RNA was then isolated and hybridized to a radioactive DNA probe that overlaps the 5' end of X. laevis rRNA; endogenous rRNA of the X. borealis oocytes does not hybridize to the probe. RNA/DNA hybrids were treated with S1 nuclease and protected fragments were sized by polyacrylamide gel electrophoresis. RNA made from the injected rDNA protects the same region of probe as does authentic X. laevis precursor rRNA. Thus, transcription appears to initiate on the cloned, microinjected X. laevis rDNA at the same site as is used in vivo. This synthesis is not impaired by coinjection of an amount of α-amanitin sufficient to inhibit RNA polymerase II and III; therefore the reaction is mediated by RNA polymerase I. The amount of transcription may be reproducibly quantitated and we have varied a number of parameters in order to maximize transcriptional expression of the injected rDNA. Eight independently isolated X. laevis rDNA clones as well as several subcloned initiation regions of these genes are all accurately transcribed at approximately equal efficiency. This assay should facilitate analysis of several aspects of rRNA transcription, including deleniation of the Xenopus RNA polymerase I promoter location.

INTRODUCTION

Major advances in understanding the molecular mechanism of transcription by RNA polymerase form III (RNAP III) and RNA polymerase form II (RNAP II) have recently been made (1-5). These advances are based on the availability of systems where RNAP III and RNAP II faithfully initiate transcription on cloned DNA and where this transcription can conveniently be assayed by polyacrylamide gel electrophoresis (6). Since cloned DNA can conveniently and systematically be altered in vitro, these transcription systems are especially suited to assay the effect of mutated templates.

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In contrast to the cases of RNAP III and RNAP II, the study of transcription by RNA polymerase form I (RNAP I) has lagged, due largely to the lack of a versatile transcriptional assay for cloned rRNA genes. Many RNAP I transcription studies have focused on Xenopus; X. laevis was the only vertebrate where the site of rRNA transcriptional initiation was precisely known (7), and this information is necessary in order to verify that initiation on cloned rDNA is accurate. Several previous attempts to demonstrate transcription on purified rDNA were unsuccessful (8,9); others were suggestive but lacked a sufficiently sensitive assay to confirm accurate initiation (10,11). The most promising Xenopus RNAP I transcription system to date was developed by Trendelenburg and Gurdon (12). They injected cloned X. laevis rDNA into X. laevis oocytes and subsequently spread the oocyte chromatin for electron microscopy by the Miller procedure (13). A number of examples of plasmid DNA molecules bearing the characteristic length rRNA transcription complexes were observed, indicating that correct initiation and termination were indeed occurring. However, these transcription complexes are seen infrequently, even under optimized conditions (14) and therefore this method has not evolved as a routine transcriptional assay. Moreover, this electron microscopic assay does not permit precise determination of the transcriptional initiation site.

In order to routinely assay correct initiation on rDNA injected into Xenopus oocytes, biochemical detection methods similar to those used in RNAP III and RNAP II systems are required. Since there is a large amount of rRNA synthesis in the oocyte from endogenous genes, a limited amount of transcription from the injected rDNA would be undetected by the direct RNA labelling method used to assay transcription mediated by RNAP III (15). Similarly, the large amount of endogenous rRNA in the oocyte would interfere with direct analysis of transcription from injected genes by S1 nuclease analysis, as has recently been used to assay transcription mediated by RNAP II (5). In order to avoid these problems, we have taken advantage of the sequence divergence of rDNA between X. laevis and X. borealis (16,17); using S1 nuclease mapping we are able to detect limited quantities of transcripts from X. laevis rRNA genes microinjected into X. borealis oocytes. This method is a sensitive, accurate, and convenient biochemical assay for transcriptional initiation on Xenopus rRNA genes.

METHODS

Oocyte injections: Sections of ovary were surgically removed from adult

X. borealis females anesthetized with 0.1% tricane methanesulfonate (Sigma, also called MS222). Oocytes were disected from the membrane with tweezers in modified Barth's buffer (18), pooled, aligned on a nylon mesh (Tetco) in a 3 cm petri dish and centrifuged at 300 X g for 5 minutes. ∿30 nl of a 200-500 μg/ml DNA solution (generally in 10 mM Tris-HCl (pH 7.9) 0.1 mM EDTA) was manually microinjected into the nucleus of each of 20 mature oocytes (19,20). Where indicated, 1 μg/μl of α-amanitin (Boehringer) was coinjected with the DNA. Oocytes were generally incubated for 6-8 hours in modified Barth's buffer (18) at 20°C, then washed in phosphate buffered saline and processed for the extraction of total RNA (21). Initially injections were verified by microinjection of bromphenol blue and disection of the nucleus; nuclei are not visible in these centrifuged oocytes.

S1 nuclease analysis: In order to prepare the hybridization probe, a 180 bp Hinf I fragment of plasmid pXlr14c that contains the rDNA initiation site (7) was isolated from a 6% polyacrylamide gel and labelled at its 5' termini (22) with γ-^{32}P-ATP and polynucleotide kinase (PL Biochemicals). Complementary DNA strands were then separated on a 8% polyacrylamide gel (30:1 acrylamide to bisacrylamide, 50 mM TBE; 22) following denaturation in 0.1 M NaOH, and the upper strand was diffusion eluted and purified by DEAE cellulose chromatography (7). 10^{-2} pmoles of probe (generally 0.5 to 2 x 10^6 cpm/pmole) was hybridized with 8 μg of oocyte RNA (unless otherwise indicated), in 20 μl of 0.3 M NaCl, 100 mM Tris-HCl (pH 8), 10 mM $NaHPO_4$, 2 mM EDTA at 65°C overnight in a sealed capillary pipet. One Xenopus oocyte, laevis as well as borealis, yields 4 μg of RNA. Samples were then diluted fifteen fold into 30 mM sodium acetate-acetic acid (1:1; pH 4.5), 200 mM NaCl, 5 mM $ZnSO_4$ and treated with 30 U S1 nuclease (Bethesda Research Labs) for 1 hr at 20°C. Reactions were terminated by the addition of 60 l of a solution containing 1 M sodium acetate, 330 mM Tris-HCl (pH 9.5), and 67 mM EDTA, and nucleic acids were precipitated with 1 ml of EtOH. After resuspension in 95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, samples were heated to 100°C for 3 minutes and loaded onto a 4% or 8% polyacrylamide sequencing gel (20:1 acrylamide to bisacrylamide, 0.1 M TBE, 8 M urea; 23). Following electrophoresis, radiolabelled DNA was visualized by autoradiography. Quantitation of radiolabelled DNA was derived either by excising DNA bands and measuring radioactive decay by scintillation spectroscopy or by densitometric tracing of autoradiograms within their linear exposure range.

Plasmid DNAs were grown and purified as described previously (7). All work with recombinant DNA was done under P2/HV1 conditions, in accordance

with the NIH guidelines.

RESULTS

In order to elucidate the signals involved in accurate transcriptional initiation *in vivo*, we sought a system that transcribes rRNA genes under conditions as close as possible to those of an intact cell. Thus we chose to inject cloned *X. laevis* rDNA into the nuclei of *Xenopus* oocytes. We earlier used a S1 nuclease mapping technique to locate the 5' end of authentic *X. laevis* rRNA (7) and thought this procedure might also provide a simple, high resolution assay for transcription of injected cloned rDNA. In this S1 nuclease mapping, oocyte rRNA is hybridized to a 5' end-labelled DNA probe, a fragment of the rDNA coding strand that overlaps the initiation site (Fig. 1). The hybrid is treated with the single strand-specific nuclease, S1, and the protected fragment is sized by polyacrylamide gel electrophoresis. The 5' end of the RNA is thus located, since the length of the protected fragment equals the distance between the 5' end of the probe and the 5' end of the RNA (24). When this mapping is performed on total *X. laevis* oocyte RNA, a protected fragment of the expected 55 nucleotide length results (Fig. 2, lane 2). As shown earlier with purified 40S rRNA, S1 nuclease mapping actually generates a series of fragments that are 0, 1 and 2 nucleotides larger than expected (7).

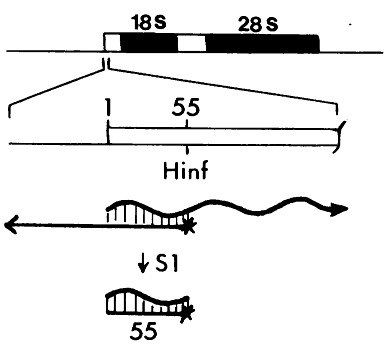

Figure 1. Outline of S1 nuclease mapping procedure.
Above is shown a region of *X. laevis* rDNA containing the 5' end of the gene. The coding strand of the Hinf I restriction fragment which spans from 125 nucleotides before to 55 nucleotides after the initiation site is isolated, labelled at the 5' end with ^{32}P (●). This probe is hybridized to RNA (〜〜>), and the hybrid is treated with S1 nuclease (below). The protected 55 nucleotide long fragment is detected by autoradiography following electrophoretic fractionation in polyacrylamide gels.

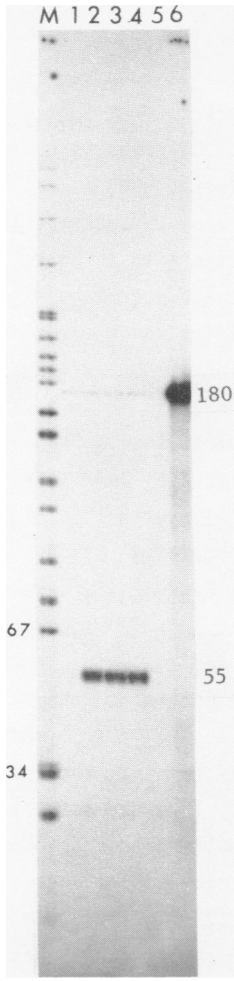

Figure 2. Use of S1 nuclease for assaying cross-species rDNA injections. RNA was hybridized to the Hinf I probe and treated with S1 nuclease. The product was denatured, fractionated on an 8% polyacrylamide gel, and detected by autoradiography. (1) RNA from two X. borealis oocytes; (2) RNA from one X. laevis oocyte; (3) RNA from two X. borealis oocytes injected with pXlr 101, a cloned entire X. laevis rDNA repeating unit; (4) RNA from two X. borealis oocytes injected with pXlr101 in 1 µg/µl α-amanitin; (5) tRNA, 10 µg; (6) untreated probe. M is Hpa II cleaved pBR322 marker.

While the S1 mapping procedure could enable detection of accurate transcriptional initiation on cloned rDNA, the abundant endogenous precursor rRNA in an injected X. laevis oocyte would hybridize to the rDNA probe as well. Hence a limited amount of rRNA made from the injected DNA would be indetectable amid the large background of endogenous rRNA. To avoid this problem, we exploited the known sequence divergence between X. laevis and X. borealis rDNA (16,17). These two frogs show only limited sequence homology around their rRNA initiation sites: there is less than 50% homology between the twenty nucleotides adjoining the 5' end of the X. laevis probe and the comparable region of X. borealis rDNA. Accordingly, the probe that is

specific for X. laevis rRNA is not protected from S1 nuclease digestion by X. borealis rRNA (Fig. 2, lane 1). Therefore, when X. laevis rDNA is injected into X. borealis oocyte nuclei, transcription of the laevis rDNA can be assayed with the laevis-specific probe without interference from borealis rRNA. As seen in Fig. 2, lane 3, the exact same length of probe is protected by RNA from the X. borealis oocytes injected with X. laevis rDNA as was protected with authentic X. laevis rRNA (Fig. 2, lane 2). We conclude that X. laevis rRNA genes initiate transcription when injected into X. borealis oocyte nuclei, and that this transcription is specific and accurate.

RNAP I is known to be responsible for rRNA transcription in vivo. In order to determine which polymerase transcribes the cloned rDNA, mixtures of DNA and α-amanitin were injected into the X. borealis nuclei. High levels (1 μg/μl) of α-amanitin do not inhibit the synthesis of X. laevis rRNA from injected rDNA, thus this transcription is indeed catalyzed by RNAP I (Fig. 2, lane 4). Control experiments demonstrate that coinjection with 1 μg/μl α-amanitin causes > 95% reduction in the specific transcription of 5S RNA genes by RNAP III and of Herpes thymidine kinase genes by RNAP II (data not shown; also see 20 and 25).

Since quantitation of RNA synthesis is important for analyzing sequence components involved in regulation of transcription, we determined the reproducibility of this rDNA transcription system and assay. The S1 nuclease assay itself is highly consistent from experiment to experiment, and is linear with the amount of input rRNA (Figs. 3A and B). Thus the quantitation rests on the reproducibility of the injections and resulting transcription reactions. With our usual injection procedure, duplicate samples are reproducible with a standard deviation of only about 20% (Fig. 3C). In this usual procedure, twenty oocytes are injected with each template DNA; about one-tenth of the pooled RNA is used for a single S1 nuclease assay. To determine the reproducibility, this injection procedure was repeated five times using five lots of twenty oocytes from a single frog. The amount of X. laevis rRNA synthesized was very similar in all five experiments (Fig. 3C). When this experiment was instead repeated using only five oocytes per injection experiment, the reproducibility was still within a factor of two (data not shown). These data are consistent with our probability of hitting the nucleus, which is approximately 60 to 70% as determined by dye injection (see Methods), and with the assumption that when rDNA is injected into a nucleus, it is transcribed at a fairly reproducible rate. In experiments where quantitation is critical, we inject lots of 50, rather than 20, oocytes

to further increase the accuracy of the results. Thus, we feel that quantitative measurements of the relative initiation frequencies of two DNA samples injected into oocytes prepared from a single female are meaningful.

In contrast, there is about a two-fold variation from frog to frog in the amount of specific transcription their oocytes support (data not shown). We therefore routinely inject a "control" DNA sample into each batch of oocytes, so that the transcriptional capacity of the experimental DNA samples can be quantitated relative to the control and independent of the quality of the oocytes. To further reduce variation, all oocytes used in an experiment are first pooled and then subdivided into lots of twenty for injection.

To optimize the amount of transcription derived from injected rDNA, we examined several parameters. As expected, the rDNA must be injected into the nucleus in order to be transcribed (Fig. 3D, lanes 1 and 3). Also, a linearized DNA is a far less efficient template than is closed circular DNA (Fig. 3D, lanes 2 and 3). A similar inefficiency of linear template has been observed for transcription by RNAP II; apparently this is due to factors other than degradation of the linear DNA (T.J. Miller and J.E. Mertz, manuscript in preparation). We have also observed that oocytes which initially appear mottled or otherwise discolored support less specific transcription than do normally pigmented ones (data not shown) and therefore only inject oocytes with normal pigmentation patterns. However, during the incubation following injection, 10 to 50% of the oocytes become partly discolored, mottled, or distended, yet such oocytes contain at least as much X. laevis rRNA as those that remain healthy (data not shown). Thus, RNA is isolated from all oocytes at the end of the incubation period.

Prior to injection, oocytes are centrifuged at low angular velocity to raise the nucleus toward the animal pole and to immobilize the oocytes on the nylon mesh. This centrifugation increases the efficiency of intranuclear injection, thus increasing the amount of transcription from the injected DNA. In contrast, when oocytes are centrifuged forcefully enough to render the nucleus visible for injections (19), the survival rate of the X. borealis oocytes decreases dramatically and they do not transcribe as efficiently as oocytes centrifuged under less forceful conditions (date not shown).

We have examined the volume and the ionic conditions of the injection media, and found that the amount of specific transcription is not highly dependent on variations in these parameters. First, most injection buffers tested, ranging from 10 mM to 250 mM total ionic strength, both with or without divalent cation, support similar amounts of transcription (Fig. 3e).

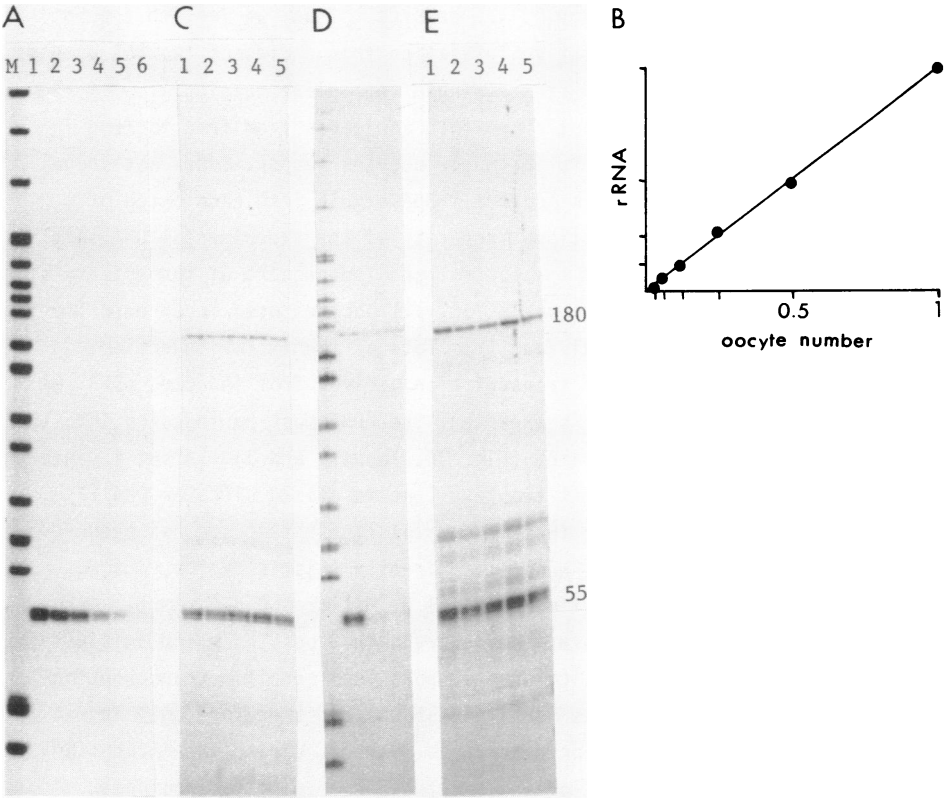

Figure 3. Quantitation and optimization of transcription.

(A,B) RNA from varying numbers of X. laevis oocytes was detected by S1 nuclease mapping. (A) M - marker; Lane 1 - 1 oocyte; Lane 2 - ½ oocyte; Lane 3 - ¼ oocyte; Lane 4 - 1/8 oocyte; Lane 5 - 1/16 oocyte; Lane 6 - 1/32 oocyte. This assay becomes nonlinear with ≥10 X. laevis oocytes per standard reaction, as the probe, 0.01 pmoles, is no longer in excess. (B) The hybridization signal measured by densitometry of the autoradiogram is proportional to the input RNA, as shown on a log-log plot.

(C) S1 nuclease mapping of the RNA produced from five independent sets of injections of plasmid pXlr14c into 20 X. borealis oocytes each. (For a map of plasmid pXlr14c, see Figure 6).

(D) S1 nuclease mapping of RNA produced after injection of plasmid pXlr14c: Lane 1 - control injection of closed circular DNA into the oocyte nucleus. Lane 2 - nuclear injection of DNA, linearized at the Eco Rl site of the pBR322 vector. The linear DNA was gel purified, hence contained no circular contaminant. We do not know whether some small fraction recircularized within the oocyte nucleus or whether linear DNA actually functions as the template. Lane 3 - injection of closed circular DNA into the oocyte cytoplasm.

(E) S1 nuclease mapping of RNA resulting from nuclear injection of plasmid pXlr14c in the following buffers: Lane 1 - 10 mM Tris-HCl (pH 7.9), 0.1 mM EDTA. Lane 2 - 10 mM Tris-HCl (pH 7.9), 1 mM $MgCl_2$. Lane 3 - one-half concentrated Barth's buffer. Lane 4 - full strength Barth's buffer

(18). Lane 5 - one and a half-fold concentrated Barth's buffer. Transcripts made in the oocytes of certain frogs, in addition to having the usual 5' end, also have minor amounts of 5' ends which map at discrete sites within a 20 nucleotide region further upstream. The reason for this result remains obscure.

However, 1X Barth's buffer reproducibly supports about 1½ times as much as the other buffers (Fig. 3e). Second, injection of up to several nuclear volume equivalents of hypotonic solution does not cause irrepairable damage to the transcription components required for accurate RNAP I function: When a fixed amount of rDNA is injected in volumes varying from 10 to 60 nl, no decrease in the amount of transcription is discernible (data not shown). Finally, the total amount of specific transcription also is not highly dependent on variation in the amount of injected DNA. As seen in Fig. 4, there is a broad range of DNA concentration within which total transcription changes only slightly. Using ∿30 nl injection volumes, the total amount of specific transcription changes by less than three-fold as the injected DNA concentration is varied from 40 to 1000 µg/ml. From this and the previous experiment, we conclude that the total amount of X. laevis rRNA synthesis obtained in injected oocytes is not highly dependent on either the exact amount or concentration of rDNA solution that is injected. Hence, small variations in the volume of rDNA solution injected into oocyte nuclei will not greatly effect the quantitative results.

The time course of the reaction has been monitored on several occasions. It is reproducibly found that the amount of X. laevis rRNA increases over the first four hours of incubation and later begins to decrease. However, the

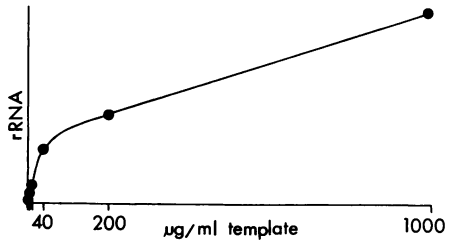

Figure 4. Dependence of transcription on injected DNA concentration. Varying amounts of plasmid pXlr14c were injected into X. borealis oocytes and the product RNA was quantitated by S1 nuclease analysis, autoradiography, and densitometry. ∿30 nl of DNA solution was injected, per oocyte, containing DNA concentrations ranging from 0.25 to 1000 µg/ml. Data are shown on a linear-linear plot.

onset of the decrease varies from experiment to experiment, as shown in the two extreme examples of Fig. 5. The reason for this variation is not known, for visually, the oocytes appear equally healthy after all of these incubation periods.

Finally, we have examined the initiation capacity of eight independently cloned X. laevis rRNA genes. All these rDNAs initiate transcription and with virtually equal efficiency (Fig. 6). This is consistent with electron microscopic observations that show that the large majority of amplified amphibian rRNA genes are capable of forming transcription units (13). Transcriptions of subcloned initiation regions of one of these genes have also been achieved; they are approximately as efficient as are full length genes (Fig. 6). The smallest subcloned region examined, plasmid pXlr14C, contains rDNA squences extending from 1050 nucleotides before the initiation site to 115 nucleotides within the rDNA transcription unit.

DISCUSSION

To study transcription of a purified gene one requires a method to effect transcription of that gene and an assay to determine the fidelity of this transcription. These two requirements are interdependent. The transcriptional method dictates what sorts of assays are applicable; the assay determines the minimum level of transcriptional specificity needed for detection and the accuracy with which this transcription can be shown to mimic the in vivo situation.

In order to investigate the mechanism of transcription by RNAP I we

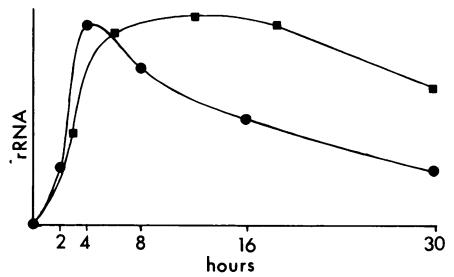

Figure 5. Time course of transcription reaction. Plasmid pXlr14c was injected into X. borealis oocytes. RNA was prepared from 20 oocytes after each of the indicated times and was quantitated as in Figure 3b and 4. Two extreme cases are shown. In both examples, the proportion of discolored oocytes did not increase from 4 to 30 hours after injection. Data are shown on a linear-linear plot.

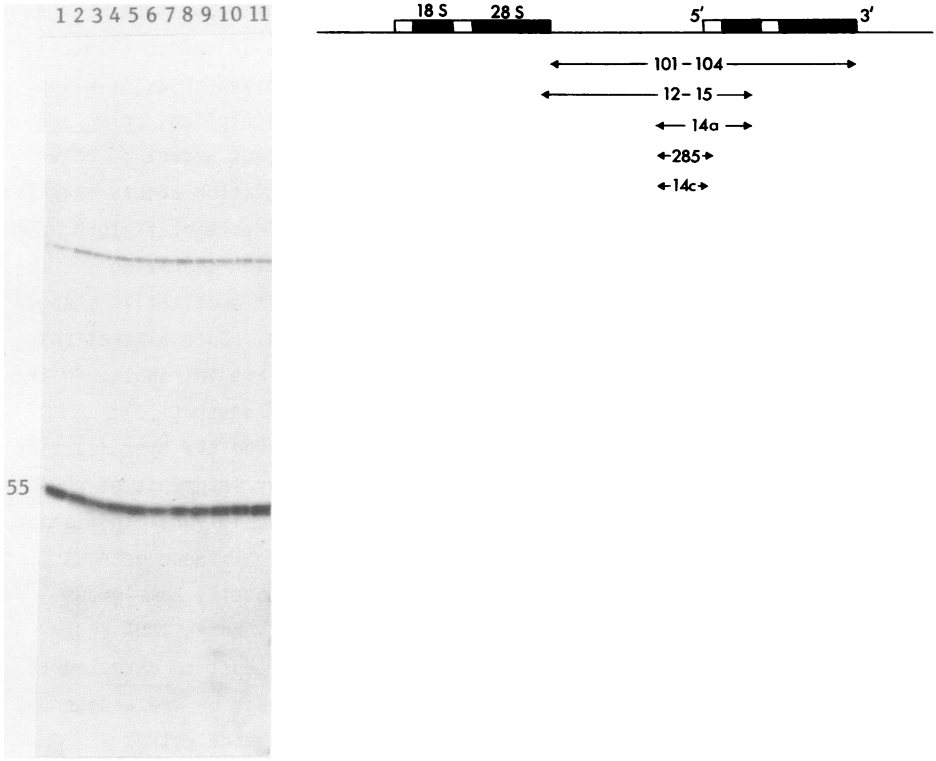

Figure 6. S1 nuclease analysis of RNA produced by 8 independent rDNA plasmids and by 3 subcloned initiation regions of plasmid pXlr14. The extent of the rDNA in these clones is illustrated at the right, aligned under a map showing two repeating units of Xenopus rDNA. Lanes 1-4: pXlr101-104 (independently isolated entire repeating units, cloned at the Hind III site (14)); lanes 5-8: pXlr12-15 (independently isolated Eco Rl fragments containing the entire nontranscribed spacer and the first 2200 bp of the transcribed region (31)); lane 9: pXlr14a (Bam Hl-Eco Rl fragment containing from 1050 bp before to 2200 bp after the initiation site subcloned from pXlr14 (7)); Lane 10: pXlr285 (subclone of pXlr14, from 1050 bp before to 285 bp after the initiation site); lane 11: pXlr14c (subclone of pXlr14, from 1050 bp before to 115 bp after the initiation site).

wanted to develop methods to effect accurate transcription of rDNA and to readily and quantitatively assay the transcription. Oocyte injection was chosen as the method to effect transcription, since it best mimics the in vivo environment of Xenopus rDNA expression. Moreover, Trendelenburg and Gurdon (12) and Bakken et al. (14) had already shown by electron microscopic methods that microinjected rDNA was able to initiate transcription in an apparently accurate manner. Recent studies, suggesting that injection

systems respond to controls for RNAP II transcription that in vitro systems do not respond to, further attest to the value of oocyte injection (4,5,26). To definitively demonstrate accurate transcription we needed an assay which was sensitive enough to detect a limited amount of transcription, selective enough to detect this transcription even amid a significant amount of other RNA synthesis, and precise enough to determine that initiation occurs exactly at the in vivo site. The precision of the assay is an important feature, for alterations of transcriptional control regions, in addition to having quantitative effects on gene expression, could also cause qualitative changes in the initiation site within the correct general region. Such alterations in exact starting sites have been observed when changes are introduced in the 5' flanking sequence of 5S RNA genes and Herpes virus TK genes (1,5).

Previous rDNA transcription assays frequently involved the hybridization of a synthesized RNA product to separated rDNA strands or fragments of rDNA and its competition with in vivo rRNA (9,10). The transcriptional initiation site used for rRNA synthesis cannot be precisely located by these methods. Moreover, transcription has routinely been effected in totally homologous systems that frequently contains large quantities of endogenous rDNA and rRNA. In these cases, transcription from the added rDNA must be detected as an increase in hybridization above the background synthesis on the endogenous template; low amounts of faithful transcription could go undetected. Trendelenburg and Gurdon (12) made a conceptual advance with the introduction of an electron microscopic assay. In this analytical method, transcription on an added small circular plasmid can clearly be distinguished from that on the much longer strands of endogenous chromatin. However, despite concerted efforts to maximize the signal (14), this assay remains cumbersome, lacks quantitative reproducibility, and is limited with respect to its precision of locating the exact initiation site.

The system described in this paper for Xenopus laevis rDNA transcription alleviates all of these problems. After injection of X. laevis rDNA into X. borealis oocytes, S1 nuclease mapping is used to sensitively, selectively, and precisely detect faithful transcriptional initiation (Fig. 1). The assay is quantitative (Fig. 2), and thus permits conclusions on the relative efficiency of different templates and transcription conditions. This S1 nuclease assay has allowed us to demonstrate that synthesis starts at precisely the same site on the injected rDNA as it does in vivo (Fig. 2) and that, in agreement with earlier results of Bakken et al. (14), transcription of injected rDNA is mediated by RNAP I (Fig. 2). Transcription requires that

the DNA is injected into the nucleus of the oocyte (Fig. 3D). Further, far more synthesis derives from a closed circular template than from a linear one (Fig. 3D). In contrast, the extent of transcription is not highly dependent on the injection buffer, injection volume, or the precise amount of injected template DNA (Figs. 3E and 4); nor is the amount of synthesis significantly affected by discoloration of the injected oocytes during the transcription reaction.

Our S1 nuclease assay has allowed us to quantitate accurate rDNA transcription in injected oocytes (Fig. 3A-C). Under standard conditions $\sim 3 \times 10^{-4}$ pmoles of authentic X. laevis rRNA 5' termini are isolated per injected X. borealis oocyte. This amounts to about 0.1 RNA molecule per injected rDNA molecule; by injecting lower amounts of DNA, up to 1 RNA molecule is made per rDNA molecule. For comparison, we measure that a X. laevis oocyte contains $\sim 10^{-3}$ pmoles of precursor rRNA molecules with intact 5' termini. These quantitations imply that transcription initiation events occur on the injected rDNA about one-third as frequently as on the endogenous rDNA genes. In contrast, in the electron microscopic assay, transcription complexes are seen far less frequently on the injected cloned rDNA than on the endogenous genes (12,14). This apparent discrepancy between the electron microscopic observations and our calculation of the relative amounts of rRNAs transcribed from the injected and the endogenous rDNA could be due to a number of causes, including the observation that the cloned rDNA molecules do not centrifuge onto the electron microscopic grid as efficiently as do endogenous rDNA molecules (A. Bakken, personal communication). Alternatively, it could be possible that injected X. laevis rDNA transcribes less efficiently in laevis oocytes used in the previous studies (12,14) than it does in X. borealis oocytes used in our studies. While this suggestion would be consistent with the known nucleolar dominance of X. laevis over X. borealis rDNA (27), control experiments using a modification of the S1 nuclease assay indicate that this is not the case (Sollner-Webb, unpublished observations).

The rDNA transcription assay reported here should prove useful for a number of studies. For instance, we already know that all rDNA genes that we have examined maintain nucleotide sequences capable of promoting transcription (Fig. 6). We also know that the entire rDNA gene is not required for proper transcriptional initiation (Fig. 6 and reference 14). We are currently defining the limits of the rDNA promoter more precisely using this transcription system to determine the transcriptional potential of rDNA molecules deleted in vitro. Preliminary results (Sollner-Webb, Schultz, Roan

and Reeder, unpublished observations) indicate that the DNA region required for transcriptional initiation resides immediately surrounding the initiation site. Thus it resides at a different location than either the region required for transcription of 5S DNA by RNAP III (1,2) or the regions required for transcription of structural genes by RNAP II (4,5,26). Further, this S1 nuclease assay has proven useful to establish an *in vitro Xenopus* rDNA transcription system (Wilkinson and Sollner-Webb, manuscript in preparation). It will be of interest to compare the nucleotide region necessary to promote *Xenopus* rDNA transcription *in vitro* with that necessary in injected oocytes since different DNA regions appear to be required for initiation by RNAP II using *in vitro* and oocyte injection systems (4,5,25). Finally, a modification of the S1 nuclease assay has allowed us (Sollner-Webb and Schultz, in preparation) to demonstrate that the transcription units which have been observed in the generally "nontranscribed spacer" of *Xenopus* rDNA, the so-called prelude complexes (28,29), do in fact initiate in a rDNA region whose sequence is >90% homologous to the normal rDNA initiation site (7,30).

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REFERENCES

1. Sakonju, S., Bogenhagen, D. and Brown, D. (1980) Cell 19, 13-25
2. Bogenhagen, D., Sakonju, S. and Brown, D. (1980) Cell 19, 27-35
3. Engelke, D., Ng, S., Shastry, B. and Roeder, R. (1980) Cell 19, 717-728
4. Grosschedl, R. and Birnstiel, M. (1980) Proc. Natl. Acad. Sci. USA 77, 7102-7106
5. McKnight, S., Gavis, E., Kingsbury, R. and Axel, R. (1981) Cell 25, 385-398
6. Birkenmeier, E., Brown, D. and Jordan, E. (1978) Cell 15, 1077-1086
7. Sollner-Webb, B. and Reeder, R. (1979) Cell 18, 485-499
8. Roeder, R., Reeder, R. and Brown, D. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 727-735
9. Hipskind, R. and Reeder, R. (1980) J. Biol. Chem. 255, 7896-7906
10. Reeder, R. and Brown, D. (1970) J. Mol. Biol. 51, 361-377
11. Beebee, T. and Butterworth, B. (1974) Eur. J. Biochem. 45, 395-406
12. Trendelenburg, M. and Gurdon, J. (1978) Nature 276, 292-294
13. Miller, O. and Beatty, B. (1969) Science 164, 955-957
14. Bakken, A., Morgan, G., Sollner-Webb, B., Roan, J., Busby, S. and Reeder, R. (1982) Proc. Natl. Acad. Sci. USA, in press
15. Brown, D. and Gurdon, J. (1978) Proc. Natl. Acad. Sci. USA 75, 2849-2853

16. Forsheit, A., Davidson, N. and Brown, D. (1974) J. Mol. Biol. 90, 301-314
17. Bach, R., Allet, B. and Crippa, M. (1981) Nucl. Acids Res. 9, 5311-
18. Gurdon, J. (1974) The Control of Gene Expression in Animal Development, Harvard University Press, Cambridge, Mass.
19. Kressman, A., Clarkson, S., Telford, J. and Birnstiel, M. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 1077-1081
20. Gurdon, J. and Brown, D. (1978) Devel. Biol. 67, 346-356
21. Probst, E., Kressman, A. and Birnstiel, M. (1979) J. Mol. Biol. 135, 709-732
22. Maxam, A. and Gilbert, W. (1980) Methods Enzymol. 65, 499-560
23. Sanger, F. and Coulsen, A. (1978) FEBS Lett. 87, 107-110
24. Sollner-Webb, B. and Felsenfeld, G. (1977) Cell 10, 537-547
25. McKnight, S. and Gavis, E. (1980) Nucl. Acids Res. 8, 5931-5948
26. Hu, S. and Manley, J. (1981) Proc. Natl. Acad. Sci. USA 78, 820-824
27. Honjo, T. and Reeder, R. (1973) J. Mol. Biol. 80, 217-228
28. Franke, W., Scheer, U., Spring, H., Trendelenburg, M. and Krohne, G. (1976) Exp. Cell Res. 100, 233-244
29. Rungger, D., Acherman, H. and Crippa, M. (1979) Proc. Natl. Acad. Sci. USA 76, 3957-3961
30. Boseley, P., Moss, T., Mackler, M., Portmann, R. and Birnstiel, M. (1979) Cell 17, 19-31
31. Botchan, P. Reeder, R. and Dawid, I. (1977) Cell 11, 599-607