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Effect of Intercalating Agents on RNA Polymerase I Promoter Selection in *Xenopus laevis*

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We have analyzed the effect of DNA intercalating agents on the transcription signals from two different *Xenopus laevis* RNA polymerase I promoters. The transcription signal from the promoter for the 7.5-kilobase rRNA precursor (the gene promoter) is unaffected over a large range of intercalating agent concentrations regardless of whether the template is injected plasmid DNA in oocytes, the amplified endogenous nucleoli of oocytes, or the endogenous chromosomes of cultured *Xenopus* kidney cells. The transcription signal from a closely related promoter located in the spacer DNA between genes (the spacer promoter) ranges between undetectable to equivalent to the gene promoter signal on different templates. The transcription signal from the spacer promoter is also differentially affected by intercalating agents relative to the gene promoter. Depending on the template, these agents can either increase or decrease the transcription signal from the spacer promoter. Fusions between the gene and spacer promoters demonstrate that intercalating agents affect transcription initiation. One explanation for these results is that the degree of supercoiling of the template DNA can differentially inhibit transcription from the spacer promoters. The different effects of intercalating agents on transcription from the spacer promoters of various templates could then be explained as differences in the degree of supercoiling present on these templates initially.

Each repeating unit of *Xenopus laevis* rDNA contains two types of RNA polymerase I promoter: the promoter for transcription of the 7.5-kilobase rRNA precursor (the gene promoter) and two or more homologous sequences located in the spacer DNA between genes (the spacer promoters). The gene promoter has been localized to the sequences between −142 and +6 relative to the transcription initiation site (5, 20). Six different spacer promoters have been sequenced and all are nearly identical to each other. However, they all differ from the gene promoter in approximately 15 positions (4a, 7, 8, 19).

There is evidence that transcription from gene and spacer promoters can be differentially regulated. In a *Xenopus* kidney cell line (4a, 6, 14) and in early *Xenopus* embryos (S. C. Pruitt and R. H. Reeder, manuscript in preparation) the transcription signals from these promoters are approximately equal. However, in several *Xenopus* tissues, including the amplified nucleoli of most oocytes, the transcription signal from the spacer promoter is only a small fraction of that from the gene promoter (4a, 6, 16). Unlike the endogenous promoters of *Xenopus* oocytes, approximately equal amounts of transcripts are detected from the gene and spacer promoters when plasmids containing rDNA are injected into these cells (4a, 6). This observation suggests that the transcription machinery of the oocyte recognizes some difference between the endogenous and injected templates.

DNA supercoiling has been implicated as one type of *cis*-acting mechanism that can affect the efficiency of transcription initiation from the promoters of both procaryotes and eucaryotes. The evidence is strongest for procaryotes, where the effect of changing superhelical density on transcription from a variety of promoters has been studied both in vitro and, by the use of DNA gyrase inhibitors and mutants, in vivo (reviewed in reference 1). In the case of the DNA gyrase promoter itself, there is evidence that the degree of DNA supercoiling serves to regulate expression from this gene (4). In eucaryotes, transcription from plasmids injected into *Xenopus* oocytes is more efficient from closed circular than from linear templates for all three RNA polymerase types (e.g., 2, 3, 10, 11a, 18). In the case of a plasmid containing four different histone genes, closed circularity of the template stimulates transcription from the different promoters to varying extents, suggesting a differential effect of DNA supercoiling on different promoters (10).

In this study we have examined the effect of DNA intercalating agents on the transcription signals from the RNA polymerase I gene and spacer promoters of *X. laevis*. The results are consistent with a differential effect of DNA supercoiling on the gene and spacer promoters, and we suggest that changes in the degree of DNA supercoiling are utilized in vivo to regulate transcription from these promoters.

MATERIALS AND METHODS

Construction of plasmids. Construction of pXlrψ52 and pXlr14G has been described previously (3a, 4a). Fusions between these two plasmids at position −35 were constructed by using a *Hga*I site present in the promoter of each plasmid. The sequence of each of these plasmids was confirmed (data not shown) by the dideoxynucleotide sequencing method (15).

Oocyte injection and nucleic acid extraction. Approximately 20 nl of Barth solution containing 6 μg of each plasmid DNA per ml was injected into the nucleus of each of 30 oocytes. The injection solution also contained 500 μg of α-amanatin per ml and ethidium bromide or chloroquine at the concentrations indicated in the text. After incubation, nucleic acids were extracted from oocytes as described previously (Pruitt and Reeder, in press).

Cell culture and nucleic acid extraction. A cultured line of *Xenopus* kidney cells (12) was maintained at room temperature in 50% L15 medium (GIBCO). Medium containing chloroquine was brought to pH 7.5 before use. To isolate

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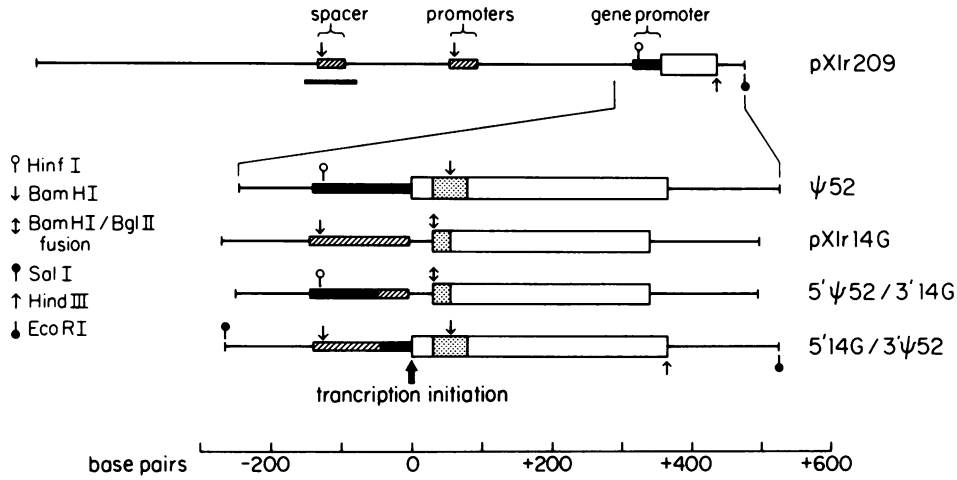


FIG. 1. Structures of plasmids containing gene and spacer RNA polymerase I promoters. Plasmid pXlr209 is the parental plasmid for each of the promoter constructs shown below it and is itself derived from pXlr14 (19) by deletion of a portion of the gene region to form a minigene body of 315 bp in length. pXlr209 contains two types of promoters: the spacer promoters, denoted with stripes, and the gene promoter, denoted by the solid black box. In pXlrψ52, the sequence 5' to −245 has been deleted and 52 bp of DNA have been inserted into the minigene body at position +31. In pXlr14 G, a 300-bp *Sma*I fragment from pXlr209 (underlined), which includes the spacer promoter, has been substituted for the gene promoter sequence of pXlrψ52 from −245 to the *Bam*HI site of the pXlrψ52 insert. This substitution shortens the transcribed portion of the plasmid by 6 bp. Plasmids pXlr −35 5'ψ52/3'14G and pXlr −35 5'14G/3'ψ52 are fusions between pXlrψ52 and pXlr14G at the *Hga*I site present in each promoter.

nucleic acids, cells were harvested and homogenized in 200 mM NaCl–10 mM Tris-hydrochloride (pH 7.5)–10 mM EDTA–1% sodium dodecyl sulfate–1 mg of proteinase K per ml. After incubation at 42°C for 1 h, lysates were extracted once with phenol and twice with 25:1 chloroform-isoamyl alcohol. Nucleic acids were precipitated with ethanol, suspended in 60 mM NaCl–10 mM Tris-hydrochloride (pH 7.5)–7 mM $MgCl_2$, and treated with 0.5 μg of DNase I per ml for 30 min at 37°C. After chloroform-isoamyl alcohol extraction, RNA was precipitated with ethanol, suspended in 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA, and stored at −20°C.

Analysis of RNA. RNA was assayed by using the primer extension protocol described in reference 13. The 88-base primer homologous to the gene between +28 and +116 and the 31-base primer homologous to the spacer between +16 and +47 were prepared in parallel, and the specific activities of the two probes were estimated from autoradiographs to vary by a factor of less than 2 to 3. Densitometry was performed as described previously (13).

Analysis of DNA. DNA recovered from oocytes was electrophoresed in a 1% agarose gel containing 5 μg of chloroquine per ml, transferred to nitrocellulose, and hybridized to nick-translated pBR322 as described previously (11a).

RESULTS

Effect of DNA intercalating agents on injected gene and spacer RNA polymerase I promoters in oocytes. The structure of the canonical rDNA spacer of *X. laevis* is represented by plasmid pXlr209 (Fig. 1) (13). A large portion of the sequence for the 7.5-kilobase rRNA precursor has been deleted from this plasmid between 115 base pairs (bp) 3' to the initiation site and 200 bp 5' to the termination site. Two derivatives from pXlr209 are used in this study. A plasmid containing only the gene promoter, pXlrψ52, was constructed by deleting the spacer sequence 5' to position −245. In addition, this plasmid has 52 bp of linker DNA inserted at position +31 (3a). In a second plasmid, pXlr14G, the spacer promoter sequence contained in the 300-bp *Sma*I DNA fragment of pXlr209 (underlined in Fig. 1) was substituted for the gene promoter of pXlrψ52 (4a). Transcripts from both pXlrψ52 and pXlr14G can be assayed simultaneously by primer extension, using an 88-base primer homologous to the endogenous sequence between +28 and +116 (13). pXlr14G yields a transcription signal that is 162 nucleotides, whereas a 168-nucleotide signal is obtained from pXlrψ52.

Analysis of transcripts from the gene and spacer promoters of plasmids injected into oocytes in the presence of increasing concentrations of ethidium bromide is shown in Fig. 2A and 3A. In the absence of ethidium bromide (lane 1), approximately equal transcription signals are detected from these promoters (4a, 6). Coinjection of ethidium bromide does not affect the 168-base transcription signal from the gene promoter of pXlrψ52 over a range of 5 to 500 μg/ml (lanes 2 through 5). In contrast, the 162-base transcription signal from the spacer promoter of pXlr14G is decreased when 5 to 100 μg of ethidium bromide per ml is coinjected (lanes 2 through 5). The minimum signal occurs at between 5 and 20 μg/ml, at which point the 162-base signal from the spacer promoter is only about 1/20 as strong as the 168-base signal from the gene promoter (Fig. 2A and 3A). As the concentration of ethidium bromide is increased, the signal from the spacer promoter of pXlr14G increases and is equal to or slightly greater than that from the gene promoter at 500 μg of ethidium bromide per ml (Fig. 2, lane 5, and Fig. 3A). At 2,000 μg of ethidium bromide per ml, transcription signals from both the gene and spacer promoters are reduced (Fig. 2A, lane 6). Figure 2B demonstrates that ethidium bromide has a similar differential effect on the transcription signals from the gene and spacer promoters when injected 6 h after the initial injection of plasmid DNA. Equal molar concentrations of a second DNA intercalating agent, chloroquine, show a differential effect similar to that described for ethidium bromide (data not shown).

To determine the effect of coinjecting ethidium bromide on the topology of the plasmid DNA recovered from oocytes, DNA was electrophoresed on a 1% agarose gel

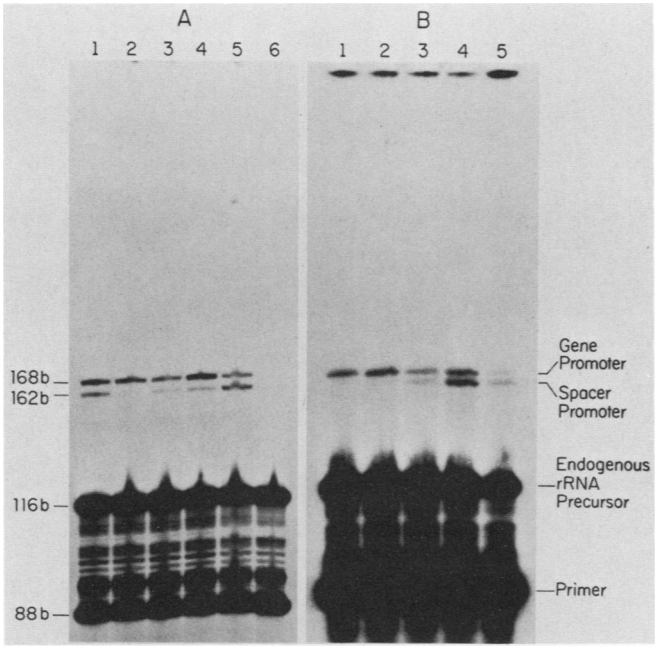


FIG. 2. Effect of ethidium bromide on transcription signals from injected gene and spacer promoters in oocytes. (A) Equal molar concentrations of gene (pXlrψ52) and spacer (pXlr14G) promoter plasmids were coinjected with increasing concentrations of ethidium bromide, where the ethidium bromide concentrations were: lane 1, none; lane 2, 5 μg/ml; lane 3, 20 μg/ml; lane 4, 100 μg/ml; lane 5, 500 μg/ml; lane 6, 2,000 μg/ml. Transcription was assayed by primer extension where the primer is 88 bases (b), the endogenous gene signal is 116 bases, the spacer promoter signal from pXlr14G is 162 bases, and the gene promoter signal from pXlrψ52 is 168 bases. (B) The gene and spacer promoter plasmids were first injected in the absence of ethidium bromide. After a 6-h incubation, oocytes were given a second injection with ethidium bromide, where lane 1 is 5 μg/ml, lane 2 is 20 μg/ml, lane 3 is 100 μg/ml, lane 4 is 500 μg/ml, and lane 5 is 2,000 μg/ml, and were incubated for an additional 12 h before assaying for transcription.

containing 5 μg of chloroquine per ml, transferred to nitrocellulose, and hybridized to nick-translated pBR322 DNA. The degree of negative superhelicity of the recovered DNA is affected by injection of ethidium bromide, particularly at the highest concentrations (data not shown). However, this result is difficult to interpret since the topology of the recovered DNA reflects a sum of several interrelated factors, including the density of bound nucleosomes, the concentration of bound ethidium bromide, and the degree to which the torsional stress induced by these agents is relaxed by DNA topoisomerase. Also, the average DNA topology is measured in this assay whereas transcription occurs on only a small fraction of the injected plasmid DNA.

Effect of DNA intercalating agents on endogenous gene and spacer RNA polymerase I promoters. The reduced level of transcripts from the spacer promoters, but not the gene promoters, of injected plasmids in the presence of low concentrations of intercalating agents mimics the differential transcription observed from the endogenous promoters of most oocytes. This result suggests that DNA supercoiling could provide the mechanism by which endogenous spacer promoters are repressed in the amplified nucleoli of these cells. If this is the case, the observation that coinjection of sufficiently high concentrations of intercalating agents causes the signal from the spacer promoters of injected plasmids to return to approximately the level observed when plasmids are injected in the absence of intercalating agents predicts that injection of sufficiently high concentrations of these agents into oocytes would also stimulate transcription from the endogenous spacer promoters.

Transcription from the endogenous gene promoter can be assayed by primer extension, using the 88-base primer described previously. Extension of this primer on transcripts from the endogenous gene promoter gives a signal band of 116 bases (Fig. 4, lanes 1 through 6). To assay transcription from the endogenous spacer promoters, a second 31-base primer extension probe, homologous to the spacer between +16 and +47 relative to the transcription initiation site, was prepared. Extension of this primer on transcripts from the endogenous spacer promoter gives a signal band of 47 bases (Fig. 4, lanes 7 through 12). Comparison of lanes 1 and 7 confirms that in the absence of intercalating agents transcripts from the spacer promoter are present at <1/100 the concentration of transcripts from the gene promoter. Consistent with the effect on injected plasmids, ethidium bromide has no effect on the concentration of transcripts from the endogenous gene promoter when injected at concentrations of 5 to 500 μg/ml (lanes 2 through 5). As predicted from the effect on injected plasmids, injection of ethidium bro-

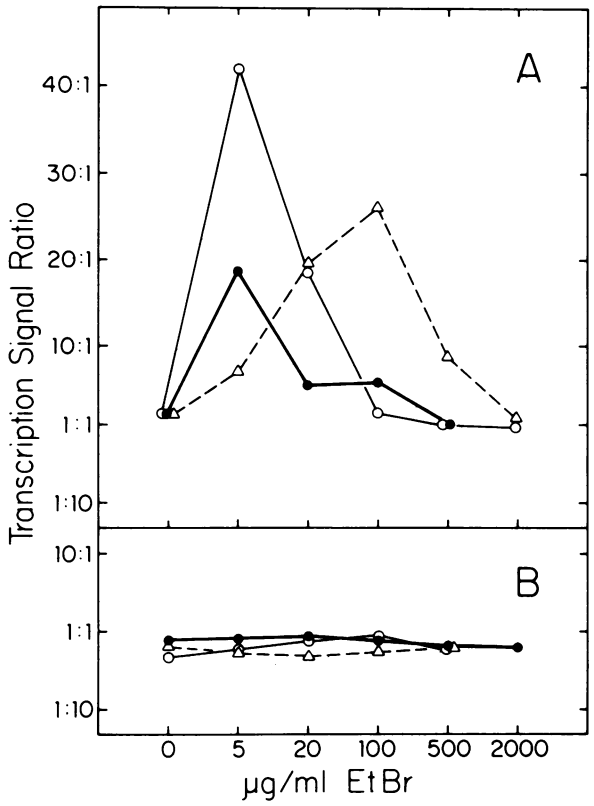


FIG. 3. Ratios of transcription signals from injected gene, spacer, and fusion promoters in oocytes. Transcription signals were quantitated by densitometry. (A) Ratio of the transcription signal from the gene promoter of pXlr52 to that from the spacer promoter of pXlr14G at increasing concentrations of ethidium bromide (EtBr) for three experiments. (B) Ratio of the transcription signal from the fusion promoter of pXlr −35 5′ψ52/3′14G to than from the fusion promoter of pXlr 5′14G/3′ψ52 at increasing concentrations of ethidium bromide for three experiments. The transcription signal ratios determined for different ethidium bromide concentrations in the same experiment are connected with a line.

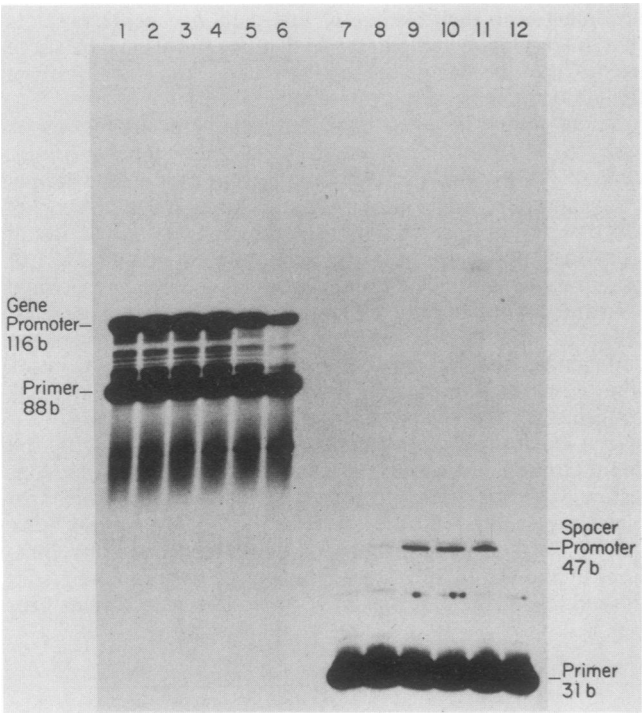


FIG. 4. Effect of ethidium bromide on transcription signals from the endogenous gene and spacer promoters of oocytes. *Xenopus* oocytes were injected with the same ethidium bromide concentrations as used in Fig. 2A, incubated for 12 h, and assayed for transcription by primer extension. Transcription signals from the endogenous gene promoter are shown in lanes 1 through 6, where the primer band is 88 bases (b) and the gene promoter signal is 116 bases. Transcription signals from the spacer promoter are shown in lanes 7 through 12, where a 31-base primer homologous to the +16 to +47 position of the spacer transcript was used. The signal band from this primer is 47 bases in length.

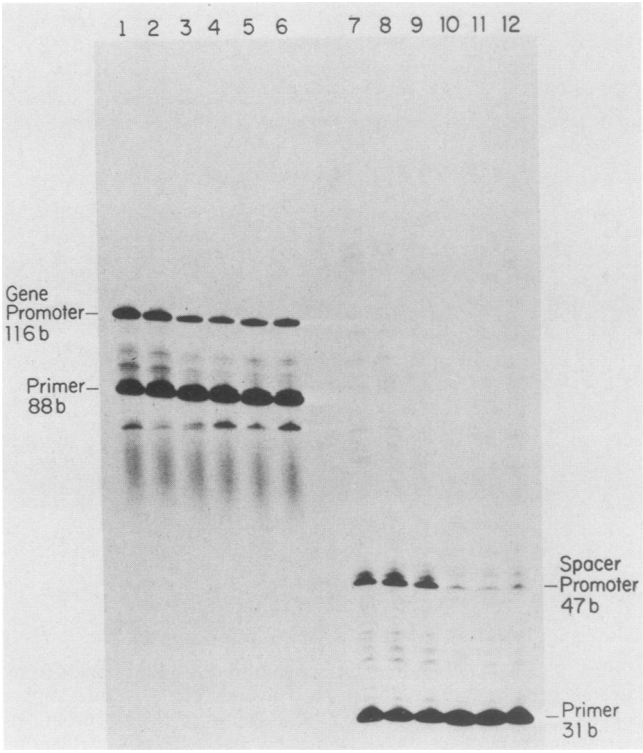


FIG. 5. Effect of chloroquine on transcription signals from endogenous gene and spacer promoters of cultured cells. A *X. laevis* kidney cell line was cultured in the absence of chloroquine (lanes 1 and 7) or in the presence of 0.5 (lanes 2 and 8), 1.0 (lanes 3 and 9), 2 (lanes 4 and 10), 2.5 (lanes 5 and 11), or 3 (lanes 6 and 12) mM chloroquine for 6 h. Transcription signals from the gene promoter are shown in lanes 1 through 6 and transcription signals from the spacer promoter are shown in lanes 7 through 12, as described in the legend to Fig. 4. b, Bases.

mide causes the concentration of transcripts from the endogenous spacer promoters of the same oocytes to increase by a factor of >20. Unlike the case for the injected plasmid DNA, this stimulation occurs over the range of 5 to 20 μg of ethidium bromide per ml (lanes 8 and 9). Although transcripts from the endogenous spacer promoters only reach a concentration of approximately 5% of the gene promoter transcripts, this result nonetheless suggests that a change in torsional stress on the endogenous ribosomal genes can activate transcription from the spacer promoters.

Transcription from the spacer promoters of endogenous ribosomal genes is not repressed in all cell types. In a *Xenopus* kidney cell line (12) the level of transcripts from the endogenous gene and spacer promoters is approximately equal (4a, 6, 14). To examine the effect of intercalating agents on these transcriptionally active spacer promoters, cells were incubated in the presence of increasing concentrations of chloroquine for 6 h and assayed for transcripts from the gene and spacer promoters, using the primer extension assays described previously (Fig. 5). Chloroquine concentrations as high as 3 mM have no effect on the transcription signal from the gene promoter (lanes 1 through 6). In contrast, the transcription signal from the spacer promoter is unaffected between 0 and 1.5 mM (lanes 7 through 9), but is reduced by a factor of at least 20 at 2.0 mM and higher (lanes 10 through 12).

Inhibition of the spacer promoter is rapidly reversed when chloroquine is removed from *Xenopus*-cultured cells. In Fig. 6, cells were treated with 2 mM chloroquine for 6 h, washed to remove the chloroquine, and incubated for various times before assaying for transcripts. Lanes 1 through 7 confirm that chloroquine has no effect on the transcription signal from the gene promoter. Lane 9 confirms that 2 mM chloroquine reduces the transcription signal from the spacer promoter by at least a factor of 20 relative to the untreated control cells in lane 8. Lanes 10 through 14 demonstrate that this effect is fully reversed within 2 h after removal of chloroquine from the media.

Spacer promoter sequences both 5′ and 3′ to position −35 are required for sensitivity to intercalating agents. The sequence differences between the gene and spacer promoters used in this study are shown in Fig. 7. Deletion analysis has demonstrated that the sequences between −142 and +6 are sufficient for efficient promoter function in the oocyte (4a, 5, 9, 13), and under the conditions used here no transcripts were detected from a −127 deletion, and more severe deletions, at any ethidium bromide concentration (data not shown). Within this region there are 17-bp differences between these promoters. We have attempted to localize which of these sequences is required for the differential sensitivity of gene and spacer promoters to intercalating agents by substituting gene promoter sequences for those of the spacer promoter. By using a *Hga*I cleavage site present in each of these promoters, reciprocal fusions were constructed at position −35 (pXlr–35 5′ψ52/3′14G and pXlr −35 5′14G/3′ψ52; Fig. 1 and 3). The ratio of the transcription

signals from these fusion promoters after coinjection into oocytes with increasing concentrations of ethidium bromide is shown in Fig. 3B. Neither of these fusion promoters shows the inhibition characteristic of the full spacer promoter of pXlr14G when injected with ethidium bromide concentrations of between 5 and 500 μg/ml. Also, the transcription signals from the −35 fusion promoters are not inhibited by ethidium bromide when coinjected with a third plasmid containing the full-length gene promoter (data not shown). These results suggest that spacer promoter sequences both 5′ and 3′ to position −35 are required for sensitivity to ethidium bromide.

DISCUSSION

There is evidence that transcription from *Xenopus* RNA polymerase I promoters requires supercoiling of the template DNA when injected into oocytes. These promoters are transcriptionally inactive when injected in a linear conformation, and transcription ceases when circular molecules are cut in the oocyte by injection of restriction endonucleases (11a, 18). Circularity of the template per se is not sufficient to ensure transcription since circular, single-stranded RNA polymerase I promoters are replicated and ligated in the oocyte but the resulting double-stranded circular templates nonetheless remain transcriptionally inactive. However, transcription can be stimulated on these templates by injection of DNA intercalating agents (Pruitt and Reeder, submitted), suggesting that topologically constrained RNA polymerase I promoters must be placed under torsional stress for efficient transcription in the oocyte. The results presented here raise the possibility that, although both the gene and spacer RNA polymerase I promoters are stimulated by torsional stress, they also respond differentially at some superhelical densities.

In this study we have utilized DNA intercalating agents to influence the degree of superhelical stress on rDNA templates in vivo (17, 21). Although these studies are indirect, many of the alternative mechanisms by which intercalating agents could differentially influence transcription from the gene and spacer RNA polymerase I promoters can be eliminated. The possibility that the transcripts from these plasmids are differentially affected at the level of either elongation or RNA processing has been eliminated by construction of fusion promoters. In the presence of low concentrations of intercalating agents the 162-base transcript from the spacer promoter of pXlr14G is underrepresented relative to the 168-base transcript from the gene promoter of pXlrψ52. Yet the same 162-base transcript is not affected by intercalating agents when transcribed from a fusion promoter containing gene sequences 5′, and spacer sequences 3′ to position −35. This result strongly suggests that DNA intercalating agents have a differential effect on transcription initiation from injected gene and spacer RNA polymerase I promoters. The similarity of the effects of intercalating agents on the accumulation of transcripts from injected and endogenous RNA polymerase I promoters suggests that these agents act by the same mechanism on both types of template. Also, the observation that intercalating agents can cause both an increase and a decrease in the transcription signal from endogenous spacer promoters is not consistent with any simple effect on processing of the transcript from the spacer promoter. These results suggest that the differential effect of DNA intercalating agents on the endogenous gene and spacer RNA polymerase I promoters also occurs at the level of transcription initiation.

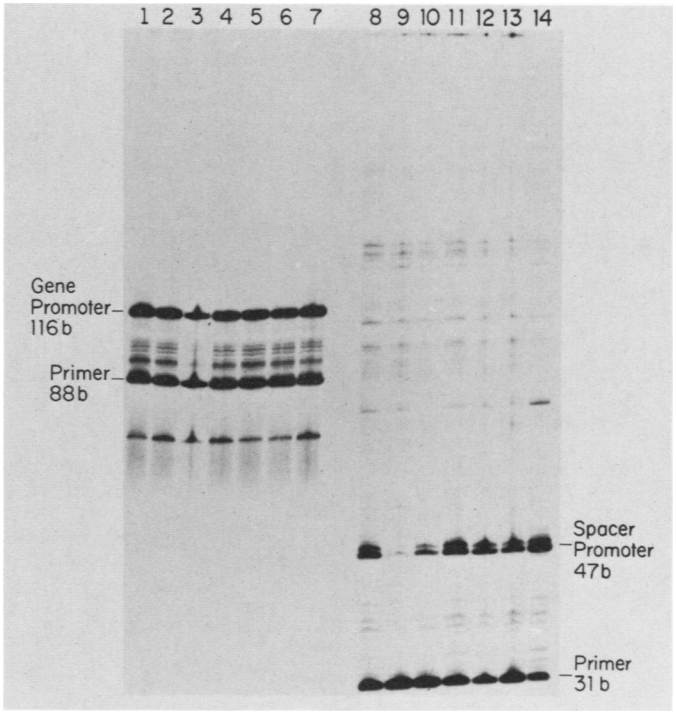
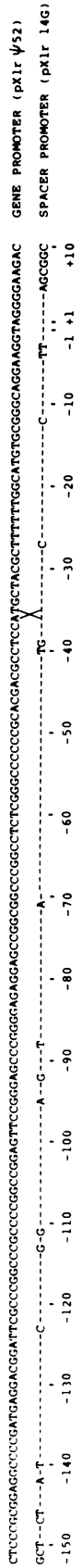


FIG. 6. Transcription signals from spacer promoters after removal of chloroquine from cultured cells. *X. laevis* cells were cultured in the presence of 2 mM chloroquine for 6 h, and cells were washed to remove the chloroquine and cultured for an additional 1 (lanes 3 and 10), 2 (lanes 4 and 11), 4 (lanes 5 and 12), 8 (lanes 6 and 13), or 16 (lanes 7 and 14) h. Transcripts from the gene (lanes 1 through 7) and spacer (lanes 8 through 14) promoters were assayed as described in the legend to Fig. 4. Lanes 1 and 8 show transcription signals from untreated control cells and lanes 2 and 9 show transcription signals from cells incubated for 6 h in the presence of 2 mM chloroquine. b, Bases.

The possibility that transcription initiation from the spacer promoter is differentially inhibited by localized binding of DNA intercalating agents within the promoter, and steric hindrance of the transcription machinery, is unlikely. Binding of intercalating agents to the spacer but not the gene promoter would require a remarkable degree of sequence specificity since these promoters differ by only 17 nucleotides out of 150 (Fig. 3). Further, the 5′-spacer/3′-gene fusion promoter, which is not sensitive to DNA intercalating agents, differs from the spacer promoter by only 4 nucleotides out of 150. Finally, a specific inhibition of the spacer promoter by steric hindrance at low intercalating agent concentrations is not consistent with the observation that transcription from this promoter resumes at higher concentrations.

The effect of intercalating agents which we consider is most likely to cause the differential inhibition of transcription from the spacer promoter is the change in torsional stress which is induced when these agents bind to topologically constrained DNA (17, 21). A differential effect of superhelical stress on transcription from the gene and spacer promoters could also explain the different levels of spacer promoter transcription observed from various templates in the absence of DNA intercalating agents. Several observations are consistent with this possibility. In the absence of intercalating agents, the spacer promoters of injected plasmids in oocytes are transcribed efficiently, whereas transcripts from the endogenous spacer promoters of oocyte are not detected. Transcription from the injected spacer promoters



is inhibited by between 5 and 20 μg of ethidium bromide per ml; however, this concentration of ethidium bromide stimulates transcription from the endogenous spacer promoters. Injection of higher concentrations of ethidium bromide does stimulate transcription from the injected spacer promoters, relative to the level at 5 to 20 μg/ml, but does not further stimulate transcription from the endogenous spacer promoters. These results could be explained if the difference in transcription of the injected and endogenous spacer promoters in the absence of ethidium bromide results from a difference in the degree of supercoiling of these templates. This initial difference would be maintained after injection of any given concentration of intercalating agent, causing the apparent shift in the concentration of these agents required to stimulate or inhibit transcription from the spacer promoters of these templates. Similarly, the observation that the endogenous spacer promoters of tissue culture cells are transcribed efficiently in the absence of intercalating agents, and are inhibited by low concentrations of these agents, could be explained if the degree of supercoiling of this template was equivalent to that of injected plasmid DNA in ooyctes. Higher levels of DNA intercalating agents than were used here might be expected to stimulate transcription from the endogenous spacer promoters of tissue culture cells; however, this possibility has not been tested.

Although the endogenous spacer promoters of *Xenopus* oocytes are stimulated by intercalating agents, the level of transcription from these promoters remains far below that from the endogenous gene promoters at any intercalating agent concentration. This result contrasts with the nearly equivalent transcription observed from the gene and spacer promoters of injected plasmids at high intercalating agent concentrations and suggests that intercalating agents can activate transcription on only a fraction of the endogenous spacer promoters. One explanation for this result is that, although the degree of DNA supercoiling of the endogenous template is sufficient to prevent transcription from the spacer promoters, some additional constraint acts to maintain the majority of these promoters in an inactive conformation (e.g., 11, 11a).

Changes in DNA supercoiling could differentially affect transcription from gene and spacer RNA polymerase I promoters by changing the conformation of binding sites for transcription factors or RNA polymerase I, similar to the influence suggested for procaryotic promoters (1, 4), or through an influence on the local chromatin structure at these promoters. Spacer promoter sequences both 5′ and 3′ to position −35 are required for the differential inhibition of transcription by intercalating agents, suggesting that DNA sequences at several locations in the spacer promoter are required for this effect. This result raises the possibility that all of the 17 nucleotide changes between the gene and spacer promoters are maintained specifically to allow a differential response of these promoters to changes in DNA supercoiling. A differential sensitivity of the gene and spacer promoters to changes in DNA supercoiling would allow the observed differential regulation of transcription from these

FIG. 7. Comparison of gene and spacer promoter sequences. The sequence of pXlrψ52 (the gene promoter plasmid) between −152 and +10 is shown. Below this sequence regions of homology to pXlr14G (the spacer promoter plasmid) are indicated by a dash and positions of divergence are indicated by the base substitution. Fusions between these promoters were made by using the *Hga*I site present in each promoter and are indicated by the large X.

closely related RNA polymerase I promoters without requiring a separate transcription factor for each promoter.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Using an S100 extract from the *X. laevis* cell line described in the text, we have assayed the effect of ethidium bromide on transcription from linearized templates containing either the spacer promoter of pXlr 14G or the gene promoter through position −160. Transcription from both templates is inhibited at ethidium bromide concentrations above 5 μg/ml and does not resume at higher concentrations. The inhibition occurs in parallel, and no differential effect is observed. This result further suggests that the differential inhibition of transcription from the gene and spacer promoters which we have described in this study does not result from a preferential binding of intercalating agents to the spacer promoter. It has not been possible to test directly for a differential effect of supercoiling on transcription in vitro due to contaminating topoisomerase activity in the extract.

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