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DNase I Footprinting Shows Three Protected Regions in the Promoter of the rRNA Genes of *Xenopus laevis*

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Extracts prepared from *Xenopus laevis* oocytes contain a protein(s) which specifically protects three discrete regions of the RNA polymerase I promoter from digestion by DNase I. Protected region I, from nucleotide +15 to nucleotide −10, spans the site of transcription initiation. Protected region II extends from nucleotide −70 to nucleotide −100 relative to initiation, falling within a 42-base-pair sequence which is homologous to the 60/81-base-pair repeated elements which occur outside of the promoter in the spacer. Protected region III is upstream of region II, from nucleotide −120 to nucleotide −140. All three regions correlate with sequences known from deletion studies to be important for promoter function. Deletion mutants which retain either region I or regions II and III together footprint normally. Deletion of region III, however, reduces but does not eliminate footprinting on region II, suggesting either that one protein binds to both regions or that the proteins which bind to these sites interact with each other.

Transcription of the ribosomal genes of *Xenopus laevis* is controlled by a promoter that extends from approximately nucleotide −142 to nucleotide +6 relative to the site of transcription initiation (19, 28). Sollner-Webb and co-workers (28) have reported that a smaller region from −7 to +6 is able to direct accurate transcription initiation by itself. Although deletion mutants coming in as far as −150 have not been analyzed by electron microscopy, loading of RNA polymerase I at the high density characteristic of ribosomal genes is observed on plasmids truncated to −320 (3). However, two lines of evidence indicate that spacer sequences upstream of −320 can influence expression of these genes. The addition of spacer sequences to ribosomal genes injected into cleaving embryos results in an increased transcription signal from the injected plasmids (5), and when ribosomal genes bearing spacers of different lengths are coinjected into oocytes, the gene bearing the longer spacer is transcriptionally dominant (20, 24). We have demonstrated that this effect in oocytes is due to tandemly repeated sequences in the spacer which are called 60/81-base-pair (bp) repeats, and that these spacer sequences have some of the properties associated with enhancers (24). In addition, Labhart and Reeder (14) have demonstrated that a block of 60/81-bp repeats can compete with a promoter when the two are coinjected into oocytes on separate plasmids. This observation suggests that the same protein(s) can bind both to these repeats and to the promoter.

The studies described above, in which sequences are manipulated and then assayed in various ways for promoter function, suggest that the promoter of the rRNA genes consists of interacting domains. This view is consistent with models for the promoter structures of the RNA polymerase II and III genes (13, 16). Further, when in vitro transcription extracts are fractionated, activities necessary for correct transcription initiation are, at least in some cases, copurified with a protein(s) which binds to specific sequences in the promoter (7–9, 21, 22). The accumulating evidence thus strongly suggests that promoter sequences identified by deletion mapping and other mutations are indeed binding sites for proteins. To examine whether the promoter domains inferred from deletion mapping of rRNA genes correlate with binding sites for sequence-specific binding proteins, we used DNase I footprinting (10). By using an unfractionated oocyte extract in conjunction with deletion mutants of the promoter region, we addressed three questions (i) Which promoter sequences are protected from DNase I digestion? (ii) Are these protected sequences completely independent binding sites, or is there evidence that the binding proteins interact? (iii) Do the 60/81-bp repeats, which have properties of enhancers and apparently compete with the promoter for factors, support stable protein binding and are thus protected? The amplification of the ribosomal genes in the oocyte and the increased concentration of transcription factors associated with the genes provides an unusual opportunity to study sequence-specific binding proteins in an unfractionated extract. All of the components necessary for transcription of the gene are present. Using this extract, we demonstrated that sequence-specific binding proteins protect discrete domains of the promoter of the RNA polymerase I gene.

MATERIALS AND METHODS

Preparation of DNA probes. Footprinting probes were prepared by labeling at a restriction endonuclease cleavage site by standard techniques (15). The labeled DNA was cut with a second restriction enzyme to produce a probe labeled at only one end. The labeled fragment was purified by electrophoresis in agarose gels and recovered by electroelution. The fragment was counted and suspended in 10 mM Tris-hydrochloride–0.1 mM EDTA at 1,000 cpm/μl; 10,000 cpm was used per reaction. This corresponds to from 2 to 10 fmol of fragment per reaction.

In all cases except those involving the 60/81-bp repeats, a previously characterized deletion mutant was used to prepare the probe (28). The deletion mutant pXlr315 Δ3′+31, which has a fully functional promoter, was used for footprinting of the full promoter because of the availability of convenient restriction endonuclease cleavage sites. Other deletion mutants used are described in the figure legends. An *Sma*I fragment containing the 60/81-bp repeats from the

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parent plasmid pXlr101A was subcloned in pBR322 in two orientations and designated 14F-A and -B (14). The original *Sma*I sites of this fragment have been replaced with *Bam*HI and *Sal*I sites.

Footprinting reactions. Oocyte homogenates were prepared from the ovaries of young *Xenopus laevis*. Frogs were sacrificed, and their ovaries were removed and rinsed thoroughly in Barths-HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) solution (11) and minced and suspended in 3 volumes Barths-HEPES solution. Collagenase (type II; Sigma Chemical Co.) was added to a concentration of 20 μg/ml, and the ovaries were gently shaken until they had dissociated into individual oocytes. Stage 3 oocytes were selected under a dissecting scope, and the number of oocytes was estimated from the volume. The oocytes were homogenized in 50 mM HEPES (pH 7.5)–50 mM NaCl–1 mM $MgCl_2$–1 mM $CaCl_2$–0.1 mM dithiothreitol–50% glycerol or in Barths-HEPES solution containing 0.1 mM dithiothreitol and 50% glycerol to yield a final volume of 2 oocytes per μl of homogenate. Micrococcal nuclease (Millipore Corp.) was added to a final concentration of 1,000 U/ml, and the homogenate was digested for 1 h at room temperature. Ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid (EGTA) was added to a final concentration of 2 mM to inactivate micrococcal nuclease and added in parallel to untreated and RNase-treated homogenates. A concentration of 10 μg of heat-treated pancreatic RNase per ml was used in the RNase-treated homogenates.

The footprinting reaction volume was 25 μl, and 2× homogenization buffer without calcium and glycerol was added to adjust the final salt conditions to equal that of the undiluted homogenate. We tested the DNase I digestion patterns of the probe alone in the presence of Ca^{2+} concentrations from 1 μM to 5 mM and Mg^{2+} concentrations from 1 μM to 10 mM (unpublished observations). We observed no changes in the relative rates of cleavage along the DNA and thus no changes in the overall appearance of the digestion pattern. DNase I (Worthington Diagnostics) was preincubated in 10 mM $CaCl_2$, and dilutions were made so that 2 μl was added to the reaction. DNase concentration ranges were 0.5 to 5 ng for probe alone and 100 to 500 ng for probe in the presence of oocyte homogenate. The addition of DNase I to the reaction was immediately followed by addition of 10,000 cpm of end-labeled DNA. The reaction was allowed to proceed for 2 min and was stopped by addition of 200 μl 0.3 M sodium acetate–5 mM EDTA–1% sodium dodecyl sulfate–10 μg of *Escherichia coli* DNA per ml. The reaction was phenol extracted and ethanol precipitated. Samples were suspended in 99% formamide and electrophoresed on a 10% polyacrylamide–7 M urea–100 mM Tris-borate sequencing gel (26). The gel was dried onto Whatman 3MM paper and exposed to Kodak XAR-5 film with an intensifying screen at −70°C.

RESULTS

Three promoter regions protected from DNase I digestion. Homogenates of *Xenopus laevis* stage 3 oocytes (6) were used as the source of footprinting proteins. The ribosomal genes are fully active at this stage of oogenesis, but these immature oocytes have not accumulated the large amounts of yolk stored by the mature oocyte. The oocyte homogenates were digested with either micrococcal nuclease or pancreatic RNase to release proteins from the endogenous DNA or RNA or both. Micrococcal nuclease was subsequently inactivated by addition of EGTA before the extract was used in the footprinting reactions. EGTA was also added to both untreated and RNase-treated homogenates to parallel the divalent cation conditions in the micrococcal nuclease-treated homogenate. A plasmid containing an intact promoter was end-labeled at the +31 position either by filling in the 3′ end of a restriction endonuclease site with radioactive nucleotides or by phosphorylating the 5′ end with [γ-^{32}P]ATP and polynucleotide kinase. Thus, binding to either DNA strand could be examined from the same starting point.

Because of endogenous nucleases in the extract, the labeled DNA fragment was not incubated with the homogenate before addition of DNase I. Instead, DNase I was added first and then the end-labeled probe was added to the prepared extracts. During the 2-min digestion, there was no detectable effect of the endogenous nuclease on digestion patterns. The results of such an experiment are shown in Fig. 1A and B. The DNase I digestion pattern of the ribosomal gene probe alone (Fig. 1A, lanes 1 and 2) was essentially identical to the digestion pattern in the presence of untreated oocyte homogenate (Fig. 1A, lane 4). We did not observe any specific effects of the untreated homogenate on DNase I digestion on any of the DNAs we used as footprinting substrates, including pBR322. When the extract was first pretreated with micrococcal nuclease, however, proteins were released which protected three regions of the DNA from DNase I digestion (Fig. 1A, lanes 5 through 8). Protected region I extended from nucleotide +15 to nucleotide −10 on the coding strand with respect to transcription initiation. Region II, from −70 to −100, fell entirely within a 42-bp region of the promoter that is homologous with the upstream 60/81-bp spacer elements. Region III spanned nucleotides −120 to −140 (Fig. 1A, lanes 9 and 10; see Fig. 3), entirely outside the homologous region. There were no obvious changes in the digestion pattern of the DNA separating regions II and III. Although regions I and II are separated by 50 bp, regions II and III are more closely spaced. Protection was observed primarily on the coding strand of the DNA (Fig. 1B). In five separate experiments, all using different extracts with three different probe preparations, we observed no protection or cleavage changes in the noncoding strand beyond those found in the experiment shown in Fig. 1. All of these extracts footprinted the coding strand. Extracts digested with RNase did not confer protection, suggesting that the protection observed in micrococcal nuclease-treated extracts results primarily from proteins released from DNA (Fig. 1A, lane 3). Only one DNase I concentration for the homogenate reactions is presented in Fig. 1. The digestion presented was most even over the ca. 200-bp region containing the promoter. DNase concentrations lower or higher than 200 ng per reaction had no effect on the appearance of the footprint itself. Variation in footprinting activity among extracts was observed, as might be expected with preparations from different frogs; that is, variations in the ability of different batches of oocytes to transcribe microinjected plasmids were routinely observed.

Comparison of DNase I digestion of the probe alone to DNase I digestion of the probe in the presence of untreated extract (Fig. 1A, lanes 1, 2, and 4) showed that the relative intensity of the more prominent bands as well as the amount of shorter fragments were decreased in the presence of crude extract. Unfortunately, this problem was exacerbated in the micrococcal nuclease-treated extracts, probably due to the release of nonspecific DNA-binding proteins. The regions of clear protection are reproducible and consistent, however. In addition to the fully protected regions, there are additional changes in cleavage pattern which are consistent

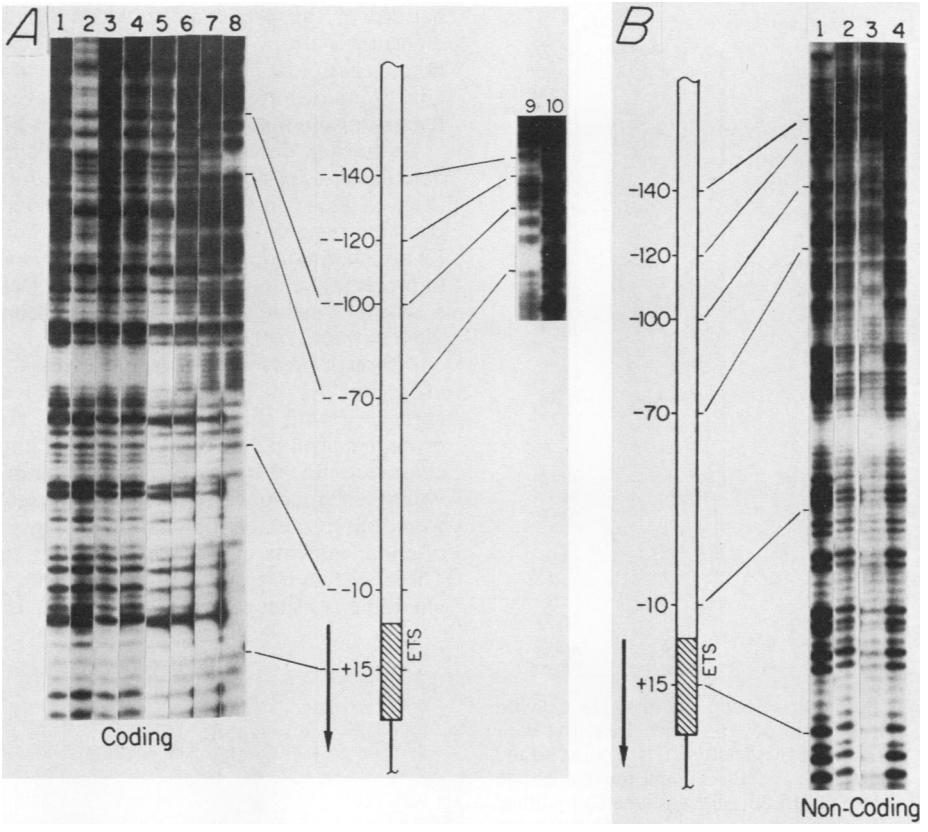

FIG. 1. Footprinting of the promoter of the *Xenopus laevis* rRNA gene. The deletion mutant pXlr315 Δ3′+31 was digested with *Hin*dIII and subsequently dephosphorylated with calf alkaline phosphatase. The coding strand was labeled via phosphorylation with [γ-^{32}P]ATP and polynucleotide kinase. The restriction endonuclease cleavage site was filled in with radioactive nucleotides by using Klenow DNA polymerase I to label the noncoding strand. ETS, External transcribed spacer. (A) Footprinting of the coding strand. Each reaction contained 10,000 cpm of labeled DNA with the following additions to the lanes: 1, 1 ng of DNase I; 2, 2 ng of DNase I; 3, 20 oocyte equivalents of RNase A-treated homogenate, 200 ng of DNase I; 4, 20 oocyte equivalents of untreated homogenate, 200 ng of DNase I; 5, 2 oocyte equivalents of micrococcal nuclease-treated homogenate, 200 ng of DNase I; 6, 5 oocyte equivalents, 200 ng of DNase I; 7, 10 oocyte equivalents, 200 ng of DNase I; 8, 20 oocyte equivalents, 200 ng of DNase I. Lanes 9 and 10 are lighter autoradiographic exposures of the −120 to −140 region: 9, 20 oocyte equivalents of micrococcal nuclease-treated homogenate, 200 ng of DNase I; 10, 20 oocyte equivalents of untreated homogenate, 200 ng of DNase I. (B) Footprinting of the noncoding strand from the same experiment. Each reaction contained 10,000 cpm of labeled DNA. Lanes: 1, 2 ng of DNase I; 2, 20 oocyte equivalents of untreated homogenate, 200 ng of DNase I; 3, 20 oocyte equivalents of micrococcal nuclease-treated homogenate, 200 ng of DNase I; 4, 20 oocyte equivalents of RNase A-treated homogenate, 200 ng of DNase I.

between experiments and which do not appear to be due to the nonspecific effects on digestion mentioned above. Because these more subtle changes cannot be unambiguously attributed to the promoter-binding proteins rather than to nonspecific binding proteins, we noted these regions (Fig. 2) but did not include them as part of the protected regions. These cleavage changes occur between ca. −50 and −70 on both strands (Fig. 1A and B).

A summary of the protection data and the sequence of the promoter and the 81-bp repeat is shown in Fig. 2. The

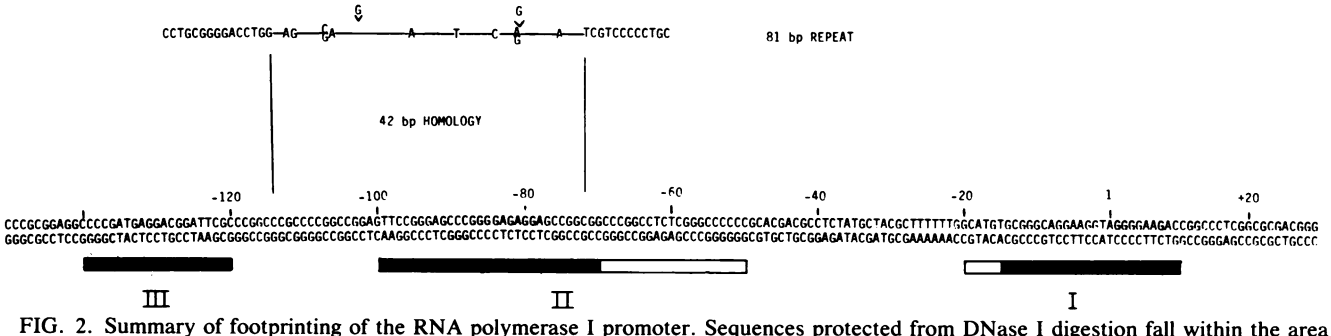

FIG. 2. Summary of footprinting of the RNA polymerase I promoter. Sequences protected from DNase I digestion fall within the area indicated with a solid black bar under the protected strand. Open bars indicate regions of consistent changes in digestion pattern, but not full protection, as described in the text.

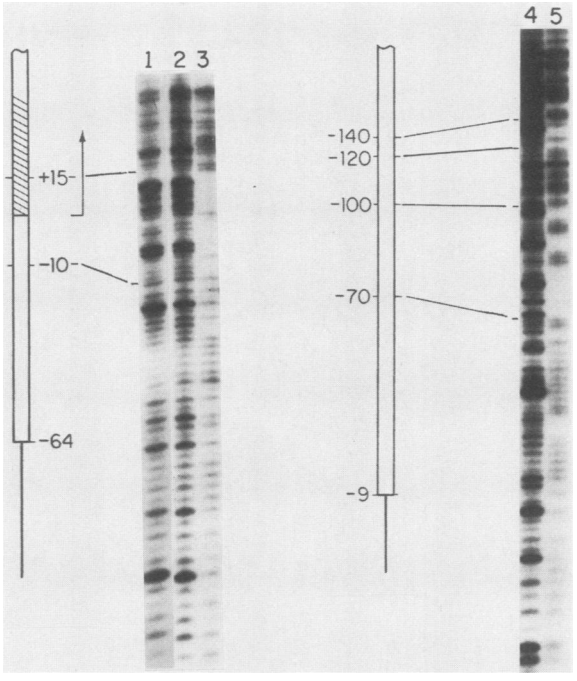

FIG. 3. Footprinting of promoter deletion mutants. The deletion mutants pXlr315 Δ3′−9 and pXlr315 Δ5′−64 were cleaved at their *Bam*HI linkers at the end points of the deletion. The coding strand of pXlr315 Δ3′−9 was labeled with [γ-^{32}P]ATP and polynucleotide kinase. The coding strand of pXlr315 Δ5′−64 was labeled by filling in the restriction site with radioactive nucleotides. Footprinting reactions contained 20 oocyte equivalents of homogenate and 10,000 cpm of DNA probe. All digestions in Fig. 1 and 3 are from one experiment. Lanes: 1, Δ5′−64, 2 ng of DNase; 2, Δ5′−64, untreated homogenate, 200 ng of DNase I; 3, Δ5′−64, micrococcal nuclease-treated homogenate; 4, Δ3′−9, untreated homogenate, 200 ng of DNase I; 5, Δ3′−9, micrococcal nuclease-treated homogenate, 200 ng of DNase I.

regions protected from DNase I, designated I, II, and III, are included within the areas marked by a solid black bar. Regions in which consistent changes in digestion pattern occur are indicated with an open bar.

Two independent domains in the promoter. To further examine these regions in the promoter protected from DNase I and to determine how they might function as promoter domains, we first used two deletion mutants as footprinting substrates. This approach was designed to test whether protected region I and the other protected regions could independently support protein binding and thus confer protection against DNase I. In the deletion mutant pXlr315 Δ5′-64, regions II and III are removed but region I is intact. The untreated homogenate had no effect on the digestion pattern compared to that with DNA alone, but the micrococcal nuclease-treated homogenate conferred specific regions of protection. Binding to region I in this mutant appeared identical to the binding observed with the whole promoter (Fig. 3). This result is consistent with the observation that the region I sequences can function as a promoter under some circumstances (28). Note that in this deletion mutant we labeled at a site upstream of the region I binding site. Comparison of Fig. 3 with Fig. 1 shows that the characteristics of the digestion patterns were virtually identical, but in inverted orientation. Similarly, footprinting was performed on deletion mutant pXlr315 Δ3′−9, in which region I is removed but both regions II and III are intact. The digestion pattern of the probe alone was virtually identical to the digestion pattern of DNA in the presence of untreated homogenate (data not shown). Removal of region I did not affect binding to regions II and III, suggesting that these two regions also constitute an independent binding site.

To further dissect the promoter domains, we used the deletion mutant pXlr315 Δ5′-115 as a footprinting substrate (Fig. 4). This construction lacks protected region III but still contains regions II and I. Thus, the contributions of region III are eliminated, but flanking sequences of the promoter 3′ to protected region II remain in place. Deletion mutants with 5′ borders inside −142 are transcriptionally inactive in in vitro extracts (28).

Although protection of region I was not affected, deletion of region III definitely reduced protection of region II compared with the protection of this region on the intact promoter or in pXlr315 Δ5′-64 (Fig. 1 and 3). Instead of the characteristic 30 bp of sequence being protected with intervening enhancements, only a few nucleotides around −100 were fully protected (compare with Fig. 3). The two clusters of enhancements characteristic of this region were absent. There was an overall decrease in cutting in this area, which would be consistent with weak binding to this site. Interest-

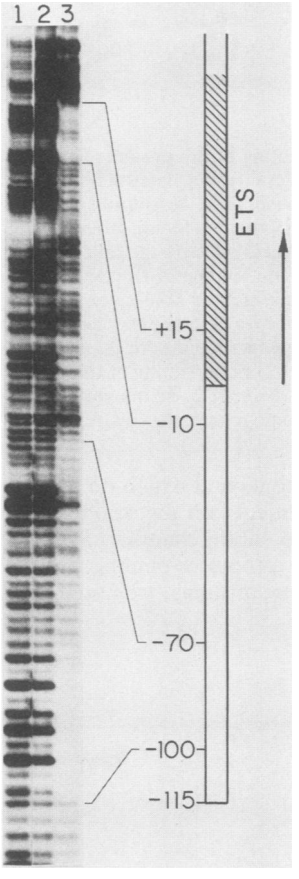

FIG. 4. Footprinting of regions I and II after deletion of region III. Deletion mutant pXlr315 Δ5′−115 was cleaved at the *Bam*HI site at the endpoint of the deletion. The restriction site was filled in with radioactive nucleotides. Footprinting reactions contained 20 oocyte equivalents of homogenate and 10,000 cpm of probe. Lanes: 1, Δ5′−115 alone, 2 ng of DNase I; 2, untreated homogenate, 200 ng of DNase I; 3, micrococcal nuclease-treated homogenate, 200 ng of DNase I.

ingly, the weakly protected region still seemed to have a border at ca. −100.

The 60/81-bp spacer elements are not protected from DNase I. The region of the promoter from −72 to −114 is closely duplicated in each 60/81-bp element of the spacer, and we have proposed a model in which these sequence elements function as factor attraction sites (24). The region of the promoter from −72 to −114 contains one of the footprint protection regions (region II, −70 to −100), and we wished to know whether binding to the related regions of the 60/81-bp elements could be detected. Since removal of region III from the promoter reduces binding at region II, we expected that binding to the spacer sequences, which do not contain a homolog of region III, would be weaker than binding to the promoter. Alternatively, it is possible that the different sequences flanking the 42-bp homologous region of the 60/81-bp repeats could positively affect binding to these elements. Under these assay conditions, however, in which footprinting was observed on the complete promoter, specific protection of the 60/81-bp elements was not detected (Fig. 5).

DISCUSSION

We showed that proteins present in oocyte homogenates can bind specifically to three distinct regions of the *X. laevis* ribosomal gene promoter. The boundaries of the protected regions are in good agreement with the boundaries of the promoter defined by Sollner-Webb et al. (28). Protected region I, from +15 to −10, contains sequences (from +4 to −11) that are conserved among different *Xenopus* species and mice (2, 17, 29). Footprinting experiments showed that binding to region I is independent of the presence of other regions of the promoter.

If regions II and III are taken together, they too can support binding independent of region I. However, since deletion of region III diminished protection of, and presumably binding to, region II, it is likely that these two regions are interdependent. The interdependence of regions II and III suggests that the promoter can be viewed as having two domains, one spanning the initiation site (region I) and a second extending from −70 to −140 (regions II and III). The interdependence of regions II and III implies that binding to these blocks of sequence is linked. It is possible that a single protein, possibly with two domains, interacts with both protected regions. In view of the size of this promoter domain (70 bp) and the rather large gap (20 bp) between the two protected regions, however, it seems more likely that two or more proteins interact with this domain. A consequence of this model is that the proteins would interact with each other as well as with the DNA, thus increasing their collective affinity for the binding site.

As described above, protected region II, from −70 to −100, occurs within an interesting sequence homology. A 42-bp sequence (from −72 to −114) is duplicated with about 80% homology in each copy of the repeated 60/81-bp elements of the spacer (4, 27). When two plasmids are injected into oocytes, the gene having more 60/81-bp elements is transcriptionally dominant (24). Because of the sequence homology between region II and the 60/81-bp elements, we hypothesized that the same factor is able to bind to both sequences. This suggestion was supported by the observation that a block of 60/81-bp elements with no adjacent promoter can effectively compete with a ribosomal gene promoter on another plasmid (14). When a block of 60/81-bp repeats is used as a footprinting substrate, however, no protection was observed under the same conditions (and same extract) for which binding to the promoter was observed. One explanation for this is suggested by the results of footprinting on a promoter deletion mutant lacking region III. Since there is no obvious counterpart to region III in the 60/81-bp elements (Fig. 2), this alone could account for the differences observed in binding to these two DNAs. At the risk of overinterpreting a negative result, however, we would point out that there are other interesting possibilities to consider.

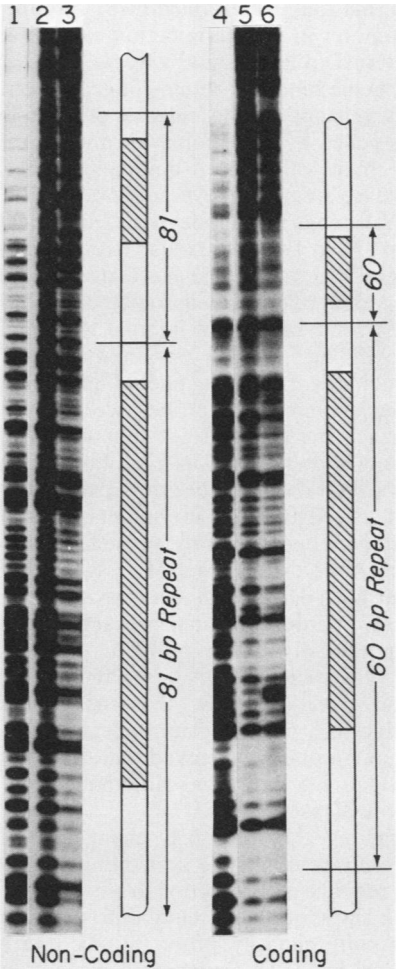

FIG. 5. Footprinting of 60/81-bp repeats. Plasmids pXlr14F-A and pXlr14F-B were labeled at their *Bam*HI sites by phosphorylation with polynucleotide kinase. Reactions contained 20 oocyte equivalents of homogenate and 10,000 cpm of probe. Lanes 1, 2, and 3 contained pXlr14F-A, and lanes 4, 5, and 6 contained pXlr14F-B. Lanes: 1 and 4, probe alone, 2 ng of DNase I; 2 and 5, untreated homogenate, 200 ng of DNase I; 3 and 6, micrococcal nuclease-treated homogenate, 200 ng of DNase I.

In our footprinting protocol, the lifetime of the protein-DNA complex muxt be 2 min, the time of digestion, to observe full protection. Thus, if binding to these elements is transitory rather than stable, it would not result in a footprint. Furthermore, the footprinting substrate is a linear molecule. Transcription of rRNA genes is severely affected by linearizing a plasmid which is transcribing in oocytes (23), and the effects of template topology on the transcription of RNA polymerase II genes has been observed previously (12, 18, 25). Perhaps more pertinent to this discussion is the

observation that only supercoiled 60/81-bp repeats compete with the promoter in oocyte injection experiments (14).

It is of interest to understand why we were able to detect sequence-specific binding proteins in crude extracts. One of the obvious reasons is the release of proteins from the endogenous genes by digestion with micrococcal nuclease. Presumably, many other DNA-binding proteins are released by this nuclease treatment. An advantage of this system is that the rRNA genes in the oocyte are specifically amplified so that they are in twofold excess over all of the chromosomal DNA of the oocyte. The associated proteins should be in a similar excess over those associated with all other DNA so that the specific binding of interest is dominant over the nonspecific binding. Another possibility is that our experimental protocol favors binding phenomena which occur very rapidly. The labeled DNA probes were not preincubated with the extract, but DNase I was first added to the extracts and then, immediately, the DNA probe. It is possible that interactions which require more time than the initial binding were not observed in our experiments. This strategy was originally adopted because of the presence of an endogenous nuclease which requires neither Mg^{2+} or Ca^{2+} in the homogenates, and also because of concern that there could be some residual micrococcal nuclease activity. Either nuclease could potentially cause problems with these experiments. We digested the DNA probes with micrococcal nuclease, however, to show that the protected regions cannot be due to a micrococcal nuclease digestion pattern (data not shown), and we checked that the digestion pattern from the added DNase I was dominant over that of the endogenous nuclease (Fig. 1A).

It is intriguing that although changes in the cleavage pattern were observed on the noncoding strand, there were no distinct regions of protection from DNase I digestion. Aiba (1) has shown that the catabolite activator protein of *Escherichia coli* primarily protects only one strand of its own promoter, but such asymmetric protection is unusual. As we argued above, if the interactions with the noncoding strand require more time or for some reason are more unstable under our assay conditions they might not be observed. Nor would they be observed if they were dependent on template topology.

The inherent weakness in an analysis using a crude extract is the inability to demonstrate a correlation between, in this case, binding and transcriptional activity. Assumption of a relationship is based on the correlations with deletion mapping of the promoter and the full transcriptional activity of the endogenous genes in the oocyte. A more detailed characterization awaits the purification of these binding proteins.

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LITERATURE CITED

1. **Aiba, H.** 1983. Autoregulation of the *Escherichia coli* crp gene: CRP is a transcriptional repressor for its own gene. Cell **32**:141–149.
2. **Bach, R., B. Allet, and M. Crippa.** 1981. Sequence organization of the spacer in the ribosomal genes of *Xenopus clivii* and *Xenopus borealis*. Nucleic Acids Res. **9**:5311–5330.
3. **Bakken, A., G. Morgan, B. Sollner-Webb, J. Roan, S. Busby, and R. H. Reeder.** 1982. Mapping of transcription initiation and termination signals on *Xenopus laevis* ribosomal DNA. Proc. Natl. Acad. Sci. U.S.A. **79**:56–60.
4. **Boseley, P., T. Moss, M. Machler, R. Portmann, and M. Birnstiel.** 1979. Sequence organization of the spacer DNA in a ribosomal gene unit of *Xenopus laevis*. Cell **17**:19–31
5. **Busby, S., and R. H. Reeder.** 1983. Spacer sequences regulate transcription of ribosomal gene plasmids injected into Xenopus embryos. Cell **34**:989–996.
6. **Dumont, J. N.** 1972. Oogenesis in *Xenopus laevis* (Daudin). 1. Stages of oocyte development in laboratory maintained animals. J. Morphol. **136**:153–179.
7. **Dynan, W. S., and R. Tjian.** 1983. The promoter-specific transcription factor SP1 binds to upstream sequences in the SV40 early promoter. Cell **35**:79–87.
8. **Engelke, D. R., S.-Y. Ng, B. S. Shastry, and R. Roeder.** 1980. Specific interaction of a purified transcription factor with an internal control region of 5S RNA genes. Cell **19**:717–728.
9. **Fuhrman, S. A., D. R. Engelke, and E. P. Geiduschek.** 1984. HeLa cell RNA polymerase III transcription factors. Functional characterization of a fraction identified by its activity in a second template rescue assay. J. Biol. Chem. **259**:1934–1950.
10. **Galas, D. J., and A. Schmitz.** 1978. DNase footprinting: a simple method for the detection of protein-DNA specificity. Nucleic Acids Res. **5**:3157–3170.
11. **Gurdon, J. B.** 1976. Injected nuclei in frog oocytes: fate, enlargement, and chromatin dispersal. J. Embryol. Exp. Morphol. **36**:523–540.
12. **Harland, R. M., H. Weintraub, and S. L. McKnight.** 1983. Transcription of DNA injected into *Xenopus oocytes* is influenced by template topology. Nature (London) **301**:38–43.
13. **Hofstetter, H., A. Kressman, and M. L. Birnsteil.** 1981. A split promoter for a eukaryotic tRNA gene. Cell **24**:573–585.
14. **Labhart, P., and R. H. Reeder.** 1984. Enhancer-like properties of the 60/81 bp elements in the ribosomal gene spacer of Xenopus laevis. Cell **37**:285–289.
15. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
16. **McKnight, S. L., and R. Kingsbury.** 1982. Transcriptional control signals of a eukaryotic protein coding gene. Science **217**:316–324.
17. **McStay, B., and A. Bird.** 1983. The origin of the rRNA precursor from *Xenopus borealis*, analyzed in vivo and in vitro. Nucleic Acids Res. **11**:8167–8181.
18. **Miller, T. J., and J. E. Mertz.** 1982. Template structural requirements for transcription in vivo by RNA polymerase II. Mol. Cell. Biol. **2**:1595–1607.
19. **Moss, T.** 1982. Transcription of cloned Xenopus laevis ribosomal DNA microinjected into Xenopus oocytes, and the identification of an RNA polymerase I promoter. Cell **30**:835–842.
20. **Moss, T.** 1983. A transcriptional function for the repetitive ribosomal spacer in *Xenopus laevis*. Nature (London) **302**:223–228.
21. **Parker, C. S., and J. Topol.** 1984. A Drosophila RNA polymerase II transcription factor contains a promoter-region-specific DNA-binding activity. Cell **36**:357–369.
22. **Parker, C. S., and J. Topol.** 1984. A Drosophila RNA polymerase II transcription factor specific for the heat-shock gene binds to the regulatory site of an hsp 70 gene. Cell **37**:273–282.
23. **Pruitt, S. C., and R. H. Reeder.** 1984. Effect of topological constraint of ribosomal DNA in *Xenopus* oocytes: comparison of plasmid and endogenous genes. J. Mol. Biol. **174**:121–139.
24. **Reeder, R. H., J. G. Roan, and M. Dunaway.** 1983. Spacer regulation of Xenopus ribosomal gene transcription: competition in oocytes. Cell **35**:449–456.
25. **Ryoji, M., and A. Worcel.** 1984. Chromatin assembly in Xenopus oocytes: in vivo studies. Cell **37**:21–32.
26. **Sanger, F., and A. Coulson.** 1978. The use of thin acrylamide gels for DNA sequencing. FEBS Lett. **87**:107–110.

27. **Sollner-Webb, B., and R. H. Reeder.** 1979. The nucleotide sequence of the initiation and termination sites for ribosomal RNA transcription in *X. laevis*. Cell **18**:485–499.
28. **Sollner-Webb, B., J. A. K. Wilkinson, J. Roan, and R. H. Reeder.** 1983. Nested control regions promote Xenopus ribosomal RNA synthesis by RNA polymerase I. Cell **35**:199–206.
29. **Wilkinson, J. A. K., K. G. Miller, and B. Sollner-Webb.** 1983. Dinucleotide primers facilitate identification of the mouse ribosomal DNA transcription initiation site. A general method for analysis of transcription by RNA polymerases I and III. J. Biol. Chem. **258**:13919–13928.