MOLECULAR AND CELLULAR BIOLOGY, Oct. 1988, p. 4282–4288
0270-7306/88/104282-07$02.00/0

Vol. 8, No. 10

Sequence Elements Essential for Function of the *Xenopus laevis* Ribosomal DNA Enhancers

CRAIG S. PIKAARD AND RONALD H. REEDER*

Basic Sciences Division, Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104

Received 28 March 1988/Accepted 17 June 1988

The intergenic spacer region of the *Xenopus laevis* ribosomal DNA contains multiple elements which are either 60 or 81 base pairs long. Clusters of these elements have previously been shown to act as position- and distance-independent enhancers on an RNA polymerase I promoter when located in *cis*. By a combination of deletion and linker scanner mutagenesis we show that the sequences essential for enhancer function are located within a 56-base-pair region that is present in both the 60- and 81-base-pair repeats. Within the 56-base-pair region one linker scanner mutation was found to be relatively neutral, suggesting that each enhancer element may be composed of two smaller domains. Each 56-base-pair region appears to be an independent enhancer with multiple enhancers being additive in effect. We review the current evidence concerning the mechanism of action of these enhancers.

The intergenic spacer regions of the ribosomal genes from *Xenopus laevis* contain sequences which have a positive effect on transcription from homologous RNA polymerase I promoters located in *cis*. This was first shown for the intact spacer by injection of genes into either oocytes (11) or cleaving embryos (2). Subsequent dissection of the spacer showed that this positive effect was largely due to repetitive elements that are either 60 or 81 base pairs (bp) long and which are arranged head to tail in blocks between the gene promoter and the duplicated spacer promoters (Fig. 1). These 60- or 81-bp elements are active when located either 5′ or 3′ to the promoter, they act at a distance, and they act in either orientation (5, 6, 15). Thus, they appear similar in function to enhancer elements that have been described for RNA polymerase II promoters.

In this paper we use a combination of deletion and linker scanner mutagenesis to delineate regions within the 60- or 81-bp elements which are essential for enhancer activity. We also examine the effect of multiple enhancers on promoter strength.

MATERIALS AND METHODS

Cloning of 60- and 81-bp elements. pXlr14 contains an *Eco*RI fragment of *X. laevis* ribosomal DNA with five spacer promoters (1) (Fig. 1). Digestion of pXlr14 with *Sma*I liberates a 740-bp fragment containing the 10 60- or 81-bp elements that are situated between the gene promoter and the proximal spacer promoter. *Bam*HI and *Sal*I linkers were attached to this 740-bp enhancer block to make the subclone pXlr14F (5). pXlr14F was sequenced on both strands by the method of Maxam and Gilbert (10) and served as the starting point for all further enhancer modifications. The sequence of the insert from pXlr14F is shown in Fig. 2.

The 740-bp *Bam*HI-*Sal*I fragment of pXlr14F was completely digested with *Pst*I and *Hae*III to yield 65- and 74-bp fragments. *Xho*I linkers were ligated onto the blunt *Hae*III ends, and the fragments were ligated into *Xho*I-*Pst*I-digested pGEM3EX (a modified version of pGEM3 in which the *Hin*dIII site of the polylinker is replaced by an *Xho*I site). Adjacent to the *Pst*I site in the polylinker is a *Sal*I site, so that inserts released by *Xho*I-*Sal*I double digestion have complimentary sticky ends for the construction of multimers.

The 65-bp fragment with attached linkers has been named EM 60 since it contains all of a 60-bp enhancer element. The 74-bp element, which contains most of an 81-bp enhancer element, has been named EM 76 since the linkers restore two nucleotides of the original sequence.

Head-to-tail repeats of the EM 60 and EM 76 elements were made by a twofold amplification method (16). Starting with a single enhancer element inserted in pGEM3EX (bounded by *Xho*I and *Sal*I sites), the plasmid was cut with *Xho*I and *Pvu*I (a single cutter in the vector sequence), and the fragment bearing the enhancer element was isolated. In a separate reaction the same plasmid was cut with *Sal*I and *Pvu*I, and again the enhancer-bearing fragment was isolated. The two isolated fragments were then ligated, resulting in the duplication of the enhancer element between the *Xho*I and *Sal*I sites of the starting plasmid. The junction between the duplicated elements is an *Xho*I-*Sal*I hybrid site which is not cut by either enzyme. Successive rounds of amplification yielded plasmids carrying two, four, and eight elements, respectively. Ligation of the appropriate plasmid halves of constructs with 2 and 8 repeats yielded plasmids with 10 repeats.

Construction of linker-scanner mutations. The 56-bp elements bearing clustered point mutations that create a novel *Xba*I site were assembled from synthetic oligodeoxynucleotides. The double-stranded oligonucleotides had *Sal*I and *Xho*I sticky ends. Head-to-tail multimers were generated by self-ligating the synthetic 60-bp repeats at 37°C in the presence of both *Sal*I and *Xho*I as described previously (16). The multimer ladder was resolved by electrophoresis, and tetramers were isolated and attached to a minigene body for assay as described below. The 56-bp element and derivatives bearing linker scanner mutations have been termed EM 0 through EM 9, and their nucleotide sequences are shown in Fig. 3.

Assay for enhancer activity. Head-to-tail polymers of var-

* Corresponding author.

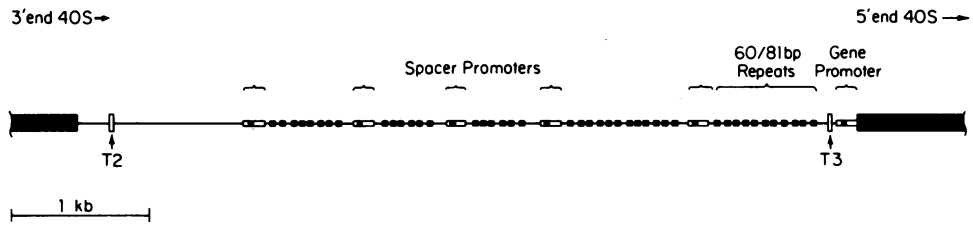

FIG. 1. Diagram of the intergenic spacer region from pXlr14. pXlr14 contains a 8,250-bp *Eco*RI fragment of *X. laevis* ribosomal DNA with an intergenic spacer that has five spacer promoters. Other regulatory elements that are indicated are the gene promoter, enhancer elements (60- or 81-bp repeats), and two sites of 3'-end formation (T2 and T3). The small black boxes indicate the region that is homologous between the enhancer elements and the promoters. kb, Kilobases.

ious enhancer elements were excised from pGEM3EX by *Xho*I-*Sal*I double digestion and ligated into the *Sal*I site at −245 bp (relative to the site of transcription initiation) of the minigene ψ40-T2 (see Fig. 3 for the structure of ψ40-T2 and a related minigene, ψ52-T2). In some cases a version of ψ40-T2 carrying a linker scanner mutation in the promoter at position −111 to −102 (LS −111/−102) (13) was used as the recipient of the enhancer elements. All enhancer constructs were assayed by injection into *X. laevis* oocytes as described previously (5). In a typical assay about 30 oocytes were injected with 0.3 fmol each of an enhancer-bearing plasmid (usually on a ψ40-T2 minigene body) and of a control plasmid (on a ψ52-T2 minigene body) in a total volume of 40 nl containing 500 μg of α-amanitin per ml. After overnight incubation, total nucleic acid was purified, and samples (generally five oocytes worth) were assayed for transcription by S1 nuclease protection by using single-stranded, end-labeled DNA probes made from the *Sal*I-*Bam*HI fragment of either ψ40-T2 or ψ52-T2 and labeled at the *Bam*HI site (7). The resulting digestion products were run on adjacent lanes of 8% acrylamide gels, autoradiographed, and traced in a densitometer.

RESULTS

Assay system for enhancer activity. The enhancers used for this study originated as a block of 10 intermixed 60- or 81-bp elements that can be excised as a 740-bp unit by digestion of the parent clone (pXlr14F) (5) with *Sma*I. This is the same enhancer block which we have used in several previous studies (1, 5, 6, 15, 18). Although the ends of this enhancer block have been sequenced (18), the internal sequence of the repetitive elements was only inferred from restriction digests (8). Therefore, as a starting point for the current work we sequenced the entire enhancer block from both strands. Only a very limited heterogeneity was found from one repeat to the next (Fig. 2). The two repeat units indicated by the arrows were chosen for further deletion and mutagenesis. In preliminary experiments we found that enhancer elements had detectable activity when assayed individually, but to obtain workable signal strength it was necessary to polymerize them. Accordingly, individual units were polymerized (by methods that gave head-to-tail arrangements; see Materials and Methods) and then inserted upstream of a ribosomal minigene. The structures of the minigenes and all mutant enhancer elements are summarized in Fig. 3.

In a typical assay, enhancer elements were inserted upstream of ψ40-T2 and injected into oocyte nuclei with an equimolar amount of ψ52-T2 (with no enhancers attached) as a competitive control. After an incubation period, the oocytes were disrupted, and RNA was purified. Transcripts were then divided into two equal samples; each was assayed with a different end-labeled S1 protection probe, each probe

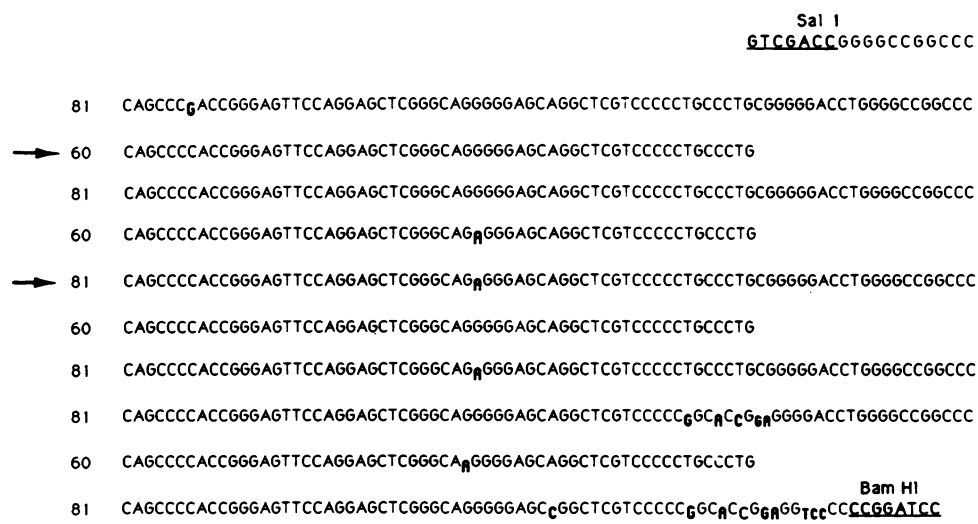

FIG. 2. Sequence of the enhancer block contained in pXlr14F. The sequence reads continuously from left to right and top to bottom. Repetitive elements are arranged under each other to align nucleotides which are repeated. Subscript indicates nucleotides which are not conserved in all repeats. Underlining indicates *Sal*I and *Bam*HI linkers that were added to the original *Sma*I fragment to facilitate cloning. The arrows indicate which elements were used for further mutagenesis.

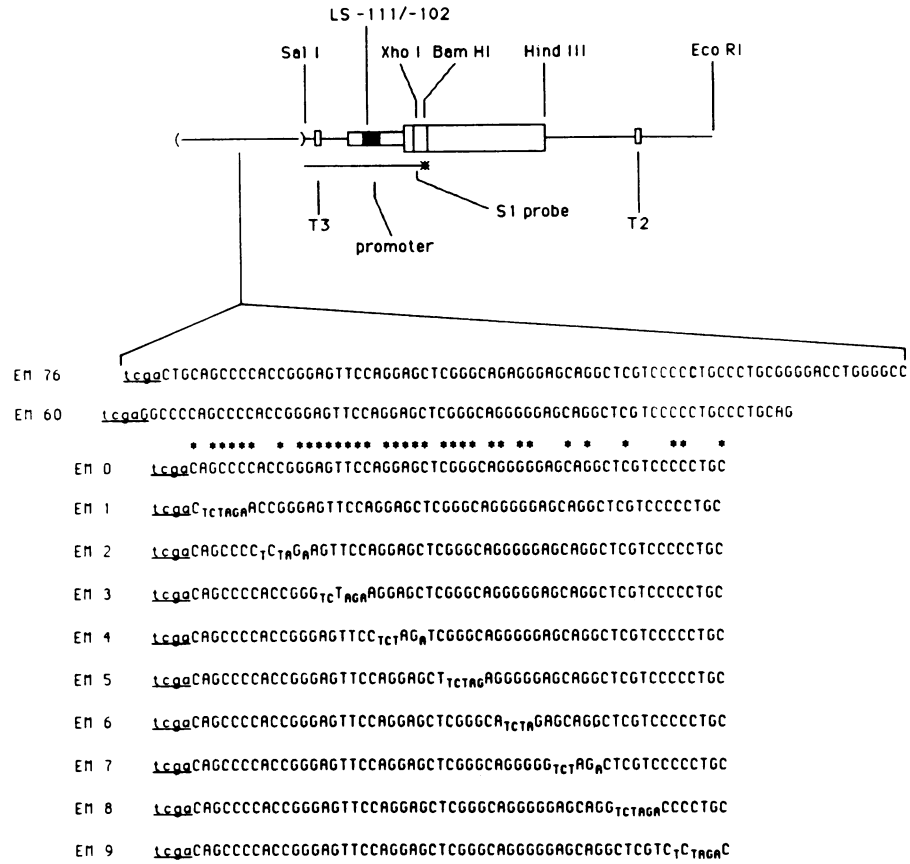

FIG. 3. Summary of enhancer mutants and diagram of the ribosomal minigene used to assay the mutants. Construction of the ribosomal minigene, ψ40-T2, has been described (9). It contains sequences from positions −245 to +115 from the 5′ end of the gene, which includes the T3 terminator, the gene promoter, and 40 bp of linker DNA (containing unique *Bam*HI and *Xho*I sites) inserted at position +31 to distinguish transcripts from the minigene from transcripts of the endogenous genes. A related plasmid, ψ52-T2, has the same structure except that 52 bp of linker DNA is inserted at position +31. The 5′ sequences are linked to a fragment from the 3′ end of the gene including the 3′ end of the 28S sequence (marked by a unique *Hin*dIII site) and site T2, which marks the 3′ end of the longest precursor identified in pulse-labeled cells. The black box marks the location of sequences in the gene promoter that are closely related to a sequence in each of the 60- or 81-bp repeats (the exact nucleotides that are conserved are marked with asterisks on the sequence below the minigene). The position of the S1 probe used to assay transcription initiation is shown just below the minigene. The sequences of various wild-type and mutated enhancer elements are shown below the minigene diagram. Nucleotides that have been changed by mutagenesis are written in subscript. Each type of enhancer element has been polymerized to various degrees as described in the text (using the linker nucleotides, which are written in lowercase letters), and each polymer was inserted at the *Sal*I site, upstream of the minigene, for assay of enhancer activity. Each polymer of a given monomer consists of exactly the sequence shown repeated head to tail for the specified number of times. In some experiments the enhancer polymers were attached to a version of the ψ40-T2 minigene that carried a LS −111/−102 in the gene promoter.

designed to detect transcripts from only one type of minigene. The protected S1 digestion products of each probe were run on adjacent lanes for comparison (Fig. 4).

Definition of the minimal enhancer element. Our first goal was to define the minimum sequence element which would retain full enhancer activity and to determine whether there were any special constraints on how this element should be arranged with its neighbors. We began by taking an EM 60 element, which contains a full 60-bp enhancer element (Fig. 3), and polymerizing it to various degrees in a head-to-tail arrangement. An autoradiograph of the assay of these EM 60 polymers (EM 60-mers) is shown in Fig. 4 (a quantitative plot of the data in Fig. 4 is shown in Fig. 7A). We observed that a single copy of EM 60 gave a detectable advantage to its adjacent promoter. After the first enhancer element, each additional element conferred the same fold increase in activity, and thus the relationship forms a straight line on a semilogarithmic plot. This implies that there is no strong cooperativity between elements; otherwise each additional element would confer a higher fold advantage than the

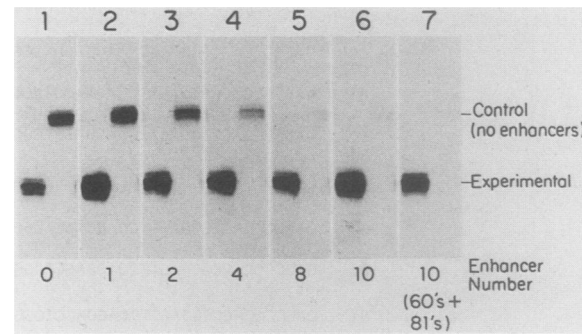

FIG. 4. Additive effect of enhancer elements on a wild-type promoter. The enhancer element EM 60 (Fig. 3) was polymerized to various degrees, attached to a wild-type promoter (ψ40-T2 minigene), and injected into oocytes with an equimolar amount of the control minigene ψ52-T2. In lane 7 the unmodified enhancer block containing a mixture of 60- and 81-bp elements was assayed in the same manner. The transcription signals from this experiment have been traced with a densitometer and are summarized in graphic form in Fig. 7A.

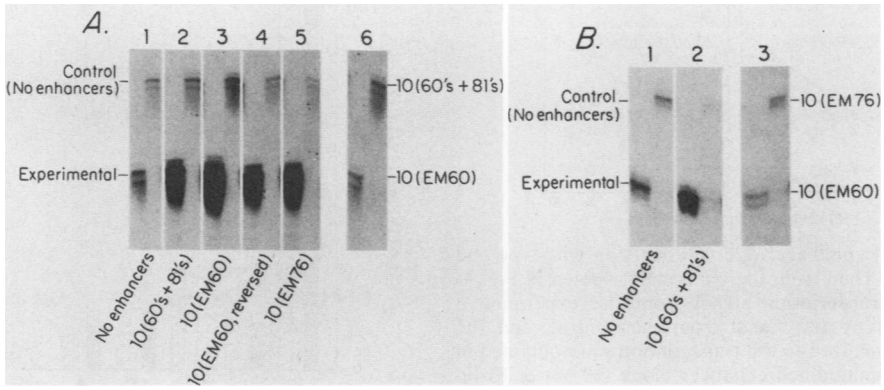

FIG. 5. EM 60 contains all detectable enhancer activity. Various enhancer elements, polymerized in blocks of 10, were assayed in competition with an enhancerless promoter and against each other. Note that EM 60, assayed in either orientation, is no less active than the block of unmodified 60- or 81-bp elements. A and B show the result of injections into separate batches of oocytes.

preceding one. Also shown in Fig. 4 and 7A is the result that 10 EM 60-mers have an activity identical to the wild-type enhancer block of 10 intermixed EM 60- and 81-mers. We draw three conclusions from this experiment. First, the effect of the EM 60-mers is additive, and there is no strong cooperativity between elements. This conclusion has previously been published by De Winter and Moss (4). Second, there is probably no critical spacing between elements, since the linkers we added to the 60-mer changed this spacing and they appear to retain full function. Third, the extra 21 bp of sequence present in each EM 81-mer has no function that we can detect in this assay, since EM 60-mers alone are as active as the wild-type mixture of EM 60- and 81-mers.

This latter conclusion is examined more carefully in Fig. 5. In this experiment 10 EM 60-mers competed against various other constructs. The most critical lanes are Fig. 5A, lane 6, and 5B, lane 3, where the EM 60-mers competed directly against a block of wild-type elements or a block of EM 76-mers, respectively. In all cases the EM 60 construct appeared to have full activity. Thus we conclude that if there is any function for the extra 21 bp of the EM 81-mer this type of assay cannot detect it.

We also examined the ability of increasing numbers of 60-bp elements to "rescue" a minigene promoter that contains an *Eco*RI restriction site inserted into the promoter at position −111 to −102. As we have shown previously, this mutation decreases basal promoter activity by 50- to 100-fold (13). Adding on a block of 10 wild-type 60- or 81-bp enhancer elements restored the mutated promoter to the same activity level as a wild-type promoter with no mutation or enhancers. Multiple elements of EM 60 were also able to rescue the mutated promoter (Fig. 6; the data in Fig. 6 are plotted in Fig. 7B). Once again we find that the relation between activity and enhancer number plots as a straight line on a semilogarithmic plot, suggesting a lack of cooperativity between the elements. A single element has a measurable effect on the promoter (Fig. 7B), and 8 to 10 elements bring the mutated promoter up to the activity of a wild-type promoter without enhancers attached. Enhancer rescue was orientation independent (Fig. 6). The fact that enhancer action was additive and orientation independent on both wild-type and mutant promoters suggests that enhancer rescue is a valid means for assaying enhancer function. With the LS −111/−102 promoter, we can obtain a reliable measure of enhancer activity with polymers of only 4 instead of 10 elements. Therefore, we used the LS −111/−102 mutated promoter to assay all further mutants of the enhancer elements.

Linker scanner mutagenesis of the basic enhancer element. A slightly truncated version of the 60-bp element was created (EM 0 in Fig. 3) and polymerized into a string of four head-to-tail repeats; its activity was compared to that of four copies of EM 60. EM 0 appeared to have full activity (Fig. 6, compare lanes 4 and 9; Fig. 7B), leading us to conclude that all detectable enhancer activity resides within the 56 bp remaining in EM 0.

We then constructed a series of linker-scanner mutants of EM 0 in which a 6-bp *Xba*I site was moved in nine increments across the active region. This resulted in a cluster of between four and six base changes for each mutant (Fig. 3). The linker scanner mutants were assayed by inserting four tandem repeats of a given mutant upstream of a minigene with the LS −111/−102 mutant promoter. All of the mutants damaged enhancer activity except for EM 6, which was nearly neutral (Fig. 8 and 9). This result suggests the

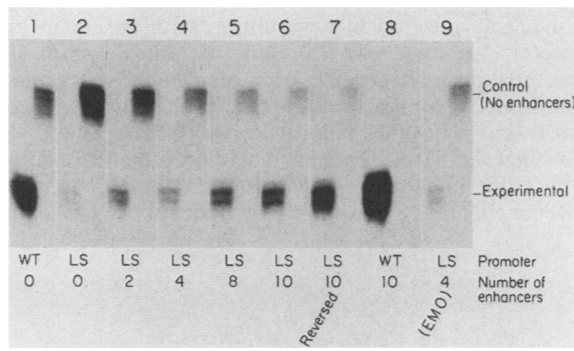

FIG. 6. Additive effect of enhancer elements on a mutant promoter (LS −111/−102). In all lanes the experimental constructs were injected with an equimolar amount of the control minigene, ψ52-T2. Experimental constructs (lanes): 1, wild-type, enhancerless promoter; 2, enhancerless mutant promoter (LS −111/−102); 3 to 6, increasing numbers of EM 60 attached to the mutant promoter; 7, 10 EM 60 units attached to the mutant promoter in the reverse orientation; 8, unmodified enhancer block (60- or 81-bp elements) attached to a wild-type promoter; 9, 4 EM 0 elements attached to the mutant promoter. The results of this experiment are summarized graphically in Fig. 7B. Note that four EM 0 elements are just as active as four of the longer EM 60 elements.

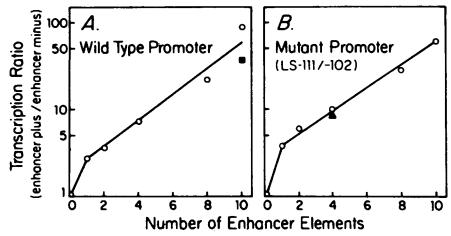

FIG. 7. Summary of enhancer additive effects on wild-type and mutant promoters. (A) Data from the experiment shown in Fig. 4. For each point the transcription signal from the experimental construct was divided by the signal from the control, and the resulting ratio was normalized to the transcription ratio obtained in lane 1. Note that the unmodified enhancer block (10 60- or 81-bp elements) cannot be distinguished from 10 EM 60 elements by this test. (B) Data from the experiments shown in Fig. 6. Data points were derived as described for panel A. Note that four EM 60 elements cannot be distinguished from four EM 0 elements by this test. Symbols: ○, EM 60 enhancer elements; ■, 10 unmodified 60- or 81-bp elements; ▲, four EM 0 elements.

possibility that each enhancer is composed of two functional domains (A and B in Fig. 9 and 10) that are separated by a short neutral region. Further work will be needed to test this hypothesis.

DISCUSSION

When the *Xenopus* ribosomal gene enhancer elements are assayed by injection into oocyte nuclei (as in this paper), it is usually necessary to inject an enhancer-bearing promoter in competition with an enhancerless promoter to reliably detect enhancer activity. This requirement for competition is apparently a peculiarity of the oocyte injection assay, possibly caused by the presence of a large excess of enhancer-binding protein in the oocyte. When the ribosomal gene enhancers are assayed by injection into cleaving embryos, they have a stimulatory effect on promoters in *cis* that is independent of competition (2, 13) and thus behave in a manner similar to that of classical polymerase II enhancers in a transient expression assay. When a promoter bearing ribosomal gene enhancers is injected in competition with an enhancerless promoter, we often observe that the transcription signal from the enhancer-bearing promoter increases while the signal from the enhancerless promoter decreases in a reciprocal fashion (Fig. 6). This reciprocal change in the signal ratio suggests that the enhancers and promoters are competing for a limiting amount of an essential transcription factor. However, in other experiments we find that as more enhancers are added, the signal from the enhancer-bearing promoter will stay constant while the enhancerless promoter signal will decrease (Fig. 4). In yet other experiments (data not shown), the enhancerless promoter signal will remain constant and the enhancer-bearing signal will increase as more enhancers are added. This variability in how the signal ratio changes seems to be a property of different batches of oocytes. Since we do not know the reason for the variability, we are reluctant to attach any theoretical significance to the way in which the signal ratio changes. However, for the three scenarios just described, when the signal ratio was

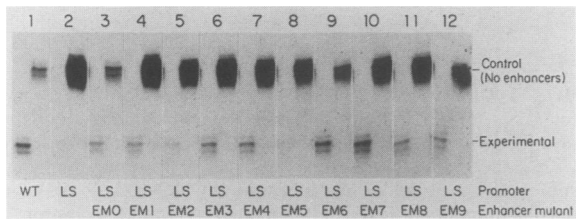

FIG. 8. Assay of linker scanner mutants of the EM 0 enhancer element. Each of the linker scanner mutants shown in Fig. 3 were polymerized into blocks of four elements, attached to the mutant (LS −111/−102) promoter, and assayed in competition with a wild-type, enhancerless promoter. The results of this plus two other independent assays are summarized in Fig. 9.

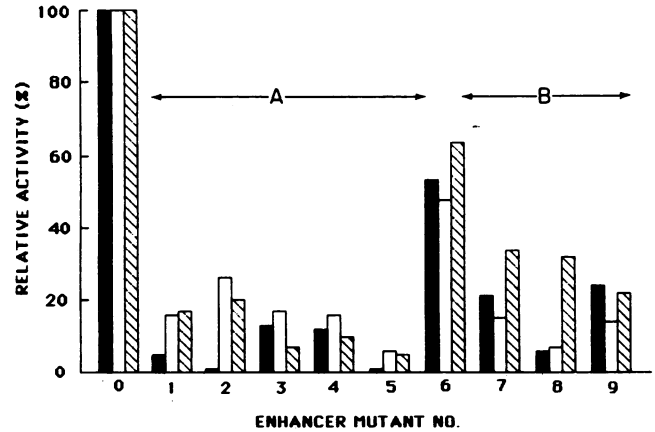

FIG. 9. Summary of three independent assays of the EM 0 linker scanner mutants. Data were taken from three experiments similar to that shown in Fig. 8. For each point, the signal from the mutant enhancer construct was divided by the signal from the wild-type (enhancerless) promoter. This ratio was then normalized to the ratio obtained with unmutated EM 0. Each different shading in the histogram represents data from an independent experiment.

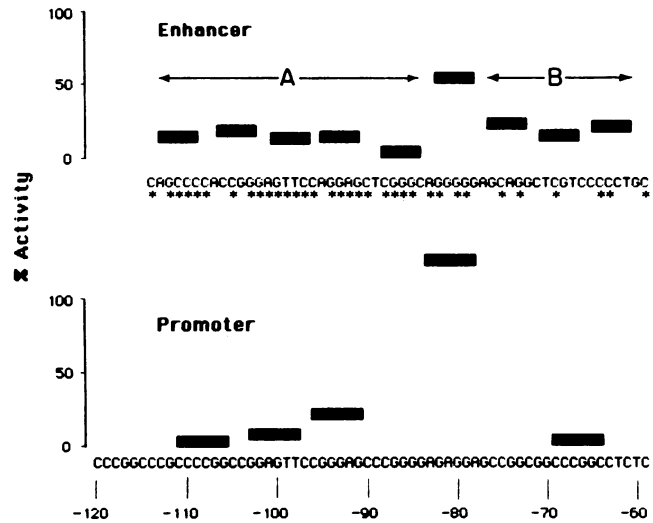

FIG. 10. Comparison of mutagenesis of the EM 0 enhancer element with mutagenesis of the enhancer homology region of the gene promoter. The nucleotide sequence of both the EM 0 and promoter region are shown with asterisks to indicate nucleotides that are conserved. Each short black bar indicates an approximate region that has been mutagenized, and the height of the bar indicates that relative activity of that particular mutant. Numbers below the promoter sequence indicate distances from the transcription initiation site.

plotted against the enhancer number (Fig. 7) we obtained a reproducible relationship that was independent of the particular oocyte batch. For this reason we think that the transcription ratio, as plotted in Fig. 7, is a valid description of ribosomal gene enhancer activity.

Two facts concerning the mechanism of polymerase I enhancer action seem fairly well established. Enhancer elements can competitively prevent transcription from a gene promoter when added in *trans* (5; B. McStay, unpublished observation), which suggests that enhancers bind an essential factor which also binds to the promoter. Also, time-of-addition experiments suggest that the enhancers act at some step early in the establishment of transcription (possibly to help establish the stable initiation complex) and that after this step they have little effect on transcription (15). Thus, we think it most likely that the enhancers help to concentrate an essential transcription factor(s) and thus increase the probability that an adjacent promoter will become activated. In harmony with this model, enhancer imbalance has been proposed as a major mechanism in the type of nucleolar dominance seen between some closely related species such as between the frogs *X. laevis* and *X. borealis* (14). Nucleolar dominance which follows the same pattern seen in *Xenopus* species (little or no maternal effect, ability to place several related species into a dominance hierarchy, and, in some of the better-studied cases, association of dominance with longer spacers) has been reported in many different plants and animals (reviewed in reference 12). By inference, this suggests that polymerase I enhancers of the *Xenopus* type may be widespread among ribosomal genes.

It has often been noted that each 60- or 81-bp enhancer element contains a region that is closely homologous with a sequence in the gene promoter. It is instructive to compare the linker scanner mutagenesis on the enhancer element with previous mutagenesis we have done on the homologous region of the gene promoter (13). Such a comparison is shown in Fig. 10. Domain A of the enhancer comprises 32 bp of sequence which turn out to be those which are most homologous with the promoter (a 25-out-of-32 match with the promoter region from positions −83 through −114). We tested three linker scanner mutations in this part of the promoter (LS −111/−102, −103/−95, and −96/−87), and all three have severe down effects on transcription (1, 8, and 19% residual activity, respectively, in oocyte injection assays). Considering the sequence homology and the functional requirement for these two regions, we hypothesize that the same protein binds to domain A of the enhancers and to region −83 through −114 of the promoter.

A relatively neutral region of the enhancer is defined by the linker scanner mutation present in EM 6. In the gene promoter, LS −83/−75 is located in an almost identical position (the four mutated bases in EM6 are in the same positions as are four of the six mutated bases in LS −83/−75) and is also neutral or, in some cases, stimulates the promoter up to twofold. Thus, domain A of the enhancers and region −83 through −114 of the promoter share sequence homology and analogous 3′ boundaries.

Domain B of the enhancers comprises 19 bp of sequence and has no obvious homology to the gene promoter (only 5 out of 19 bases are the same when compared with the promoter in the region from positions −59 through −78). A linker scanner mutation from positions −60 through −69 severely damages the promoter, and we can conclude that this also is an essential promoter element. However, it is not possible at this time to speculate whether a common factor recognizes both the enhancers and the promoter in this region.

In *X. laevis* the 60- or 81-bp elements are located in blocks between the spacer and gene promoters. It is clear that the enhancers can exert a considerable positive effect on the gene promoter in the absence of any spacer promoters, and we have not been able to obtain large positive effects by linking spacer promoters to the enhancers. However, other workers have reported synergism between the spacer promoters and the enhancers (3). The reason for this difference in results is not apparent at present.

A detectable effect on transcription is caused by the addition of one enhancer element upstream of the promoter, and the effect on transcription continues to increase as more enhancers are added (Fig. 7). Similar proportional effects have been seen with enhancers for RNA polymerase II promoters. Two examples are the metal response element of the metallothionein promoter (17) and the glucocorticoid response element of the mouse mammary tumor virus long terminal repeat (19).

ACKNOWLEDGMENTS

We thank J. Roan for technical assistance.

This work was supported by research grants to R.H.R. and a postdoctoral fellowship to C.S.P. from the National Institutes of Health.

LITERATURE CITED

1. **Botchan, P., R. H. Reeder, and I. B. Dawid.** 1977. Restriction analysis of the non-transcribed spacer of *Xenopus laevis* ribosomal DNA. Cell **11**:599–607.
2. **Busby, S., and R. H. Reeder.** 1983. Spacer sequences regulate transcription of ribosomal gene plasmids injected into *Xenopus* embryos. Cell **34**:989–996.
3. **De Winter, R. F. J., and T. Moss.** 1986. Spacer promoters are essential for efficient enhancement of *X. laevis* ribosomal transcription. Cell **44**:313–318.
4. **De Winter, R. F. J., and T. Moss.** 1987. A complex array of sequences enhances ribosomal transcription in *Xenopus laevis*. J. Mol. Biol. **196**:813–827.
5. **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.
6. **Labhart, P., and R. H. Reeder.** 1985. *Xenopus* ribosomal gene enhancers function when inserted inside the gene they enhance. Nucleic Acids Res. **13**:8999–9009.
7. **Labhart, P., and R. H. Reeder.** 1986. Characterization of three sites of RNA 3′ end formation in the *Xenopus* ribosomal gene spacer. Cell **45**:431–443.
8. **Labhart, P., and R. H. Reeder.** 1987. DNA sequences for typical ribosomal gene spacers from *Xenopus laevis* and *Xenopus borealis*. Nucleic Acids Res. **15**:3623–3624.
9. **Labhart, P., and R. H. Reeder.** 1987. A 12-base-pair sequence is an essential element of the ribosomal gene terminator in *Xenopus laevis*. Mol. Cell. Biol. **7**:1900–1905.
10. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
11. **Moss, T.** 1983. A transcriptional function for the repetitive ribosomal spacer in *Xenopus laevis*. Nature (London) **302**:223–228.
12. **Reeder, R. H.** 1985. Mechanisms of nucleolar dominance in animals and plants. J. Cell. Biol. **101**:2013–2016.
13. **Reeder, R. H., D. Pennock, B. McStay, J. Roan, E. Tolentino, and P. Walker.** 1987. Linker scanner mutagenesis of the *Xenopus laevis* ribosomal gene promoter. Nucleic Acids Res. **18**: 7429–7441.
14. **Reeder, R. H., and J. G. Roan.** 1984. The mechanism of nucleolar dominance in *Xenopus* hybrids. Cell **38**:39–44.
15. **Reeder, R. H., J. G. Roan, and M. Dunaway.** 1983. Spacer

regulation of *Xenopus* ribosomal gene transcription: competition in oocytes. Cell **35**:449–456.
16. **Rosenfeld, P. J., and T. J. Kelly.** 1986. Purification of nuclear factor I by DNA recognition site affinity chromatography. J. Biol. Chem. **261**:1398–1408.
17. **Searle, P. F., G. W. Stuart, and R. D. Palmiter.** 1985. Building a metal-responsive promoter with synthetic regulatory elements. Mol. Cell. Biol. **5**:1480–1489.
18. **Sollner-Webb, B., and R. H. Reeder.** 1979. The nucleotide sequence of the initiation and termination sites for ribosomal RNA transcription in *Xenopus laevis*. Cell **18**:485–499.
19. **Toohey, M. G., K. L. Morley, and D. O. Peterson.** 1986. Multiple hormone-inducible enhancers as mediators of differential transcription. Mol. Cell. Biol. **6**:4526–4538.