Nucleic Acids Research, Vol. 20, No. 7 1587–1592

Analysis of the rat ribosomal DNA promoter: characterization of linker-scanning mutants and of the binding of UBF

WenQin Xie, Daniel J.O'Mahony+, S.David Smith, David Lowe and Lawrence I.Rothblum*
Sigfried and Janet Weis Center for Research, Geisinger Clinic, North Academy Avenue, Danville, PA 17822-2618, USA

Received January 10, 1992; Revised and Accepted March 3, 1992

ABSTRACT

To investigate the mechanism of transcription of the rat ribosomal DNA (rDNA) promoter, a series of 23 linker-scanning mutants were constructed and assayed in transfected CHO cells and with cell-free extracts. With minor variation, the results of the *in vitro* and *in vivo* assays paralleled one another. For example, these assays demonstrated that the mutagenesis of bases from −133 to −124, and those from −106 to −101 of the rDNA promoter significantly inhibited transcription both *in vivo* and *in vitro*. Both of these sites lie within the upstream promoter element (UPE) of the rDNA promoter. Several constructs, in particular one that mutated the bases between −49 and −45, were better promoters *in vivo* than the wild-type promoter. DNAse footprinting experiments with purified UBF, an RNA polymerase I transcription factor, demonstrated the importance of the bases between −106 and −101 for the binding of that factor, providing a positive correlation between the transcription experiments and the binding of UBF to the rDNA promoter.

INTRODUCTION

The transcription initiation sites of several mammalian ribosomal RNA genes have been identified and sequenced (1,2,3). Functional analyses of the elements of the promoters of the vertebrate rRNA genes demonstrates that despite significant sequence differences, the promoters appear to consist of at least two or possibly three elements which function homologously (4,5,6). That region of the promoter (~31 to ~+6) sufficient for transcription *in vitro*, and essential for transcription *in vivo* is referred to as the core promoter element (CPE). However, when 5'-deletion mutant and wild-type promoters compete against one another under conditions where the levels of the transcription factors are limiting, a requirement for distal sequences becomes apparent (5,12). *In vivo* experiments demonstrate that constructs with only the CPE are essentially inactive (7). Two distal elements, the upstream promoter element (UPE) and a promoter-proximal terminator (referred to as T_0 in mammals, and as T_3 in *Xenopus*) have been identified (8,9,10). The UPE has been shown to be required for transcription *in vivo* (7), for elevated levels of transcription *in vitro*, and it appears to be required for the formation of the stable preinitiation complex (11,12,13). Furthermore, there is a required stereospecific alignment of the UPE and the CPE (35). Although the role of the promoter-proximal terminator is not completely understood (8,9,14), one of its functions appears to be the prevention of promoter occlusion (14,15,16). Accurate and efficient transcription initiation by RNA polymerase I requires at least two DNA-binding proteins (5,17,18). One of these factors, referred to as either SL-1 or PC-D, appears to be sufficient for transcription *in vitro*, apparently recognizes both the CPE and the UPE, and is required for species specific transcription (19,20,21). A second factor, UBF, has been isolated from several species of vertebrates (5,11,22).

UBF is one of a newly described family of DNA binding proteins whose DNA-binding domains are homologous to the HMG proteins (8,23,24,37). All vertebrate cells studied contain two mRNAs, coding for two forms of UBF, referred to as UBF1 and UBF2 (24). Mammalian forms of UBF1 and UBF2 consist of six domains: an N-terminal extension, four domains called the HMG boxes which share sequence homology to the HMG proteins and a C-terminal acid tail. Mammalian UBF2 contains a deletion of 37 amino acids in the second HMG box in comparison to UBF1 (24). UBF has been found to be a phosphoprotein (25). It has been shown that the phosphorylation of UBF is dependent upon the presence of growth factors in the media (25), and more than one domain of UBF is phosphorylated (39). It was also found that treatment of purified UBF with alkaline phosphatase reduced its ability to activate transcription in vitro (25).

UBF binds to the upstream promoter element of the mouse, rat and human 45S rRNA promoters (5,41), and to the UPE of

* To whom correspondence should be addressed

+ Present address: Center for Cardiovascular Research, Department of Medicine & Experimental Therapeutics, University College Dublin, Mater Hospital, 41 Eccles Street, Dublin 7, Ireland

the rat spacer promoter (5,38). Furthermore, analysis of the effects of deletion mutants and linker-scanning mutants of the upstream promoter elements of the rat and human promoters demonstrated that an intact UPE was required for the maximal effect of UBF *in vitro* (5,17,26). Human UBF has been shown to enhance transcription *in vitro* from human promoter constructs lacking the UPE (18). In contrast rat UBF did not enhance the transcription of similar rat promoter constructs (5). Rat UBF was shown to be required for the transcription of point mutants of the CPE of the rat rDNA promoter, referred to as UBF-rescue assays (5). However, it was not clear from those experiments if this was due to the interaction of rat UBF with the CPE or the UPE, or both. To examine this point, it was necessary to determine if we could obtain mutants within the UPE, that specifically interfered with the role of UBF in transcription in a defined manner. One way to do this would be to subject the rat rDNA promoter to linker-scanning analysis, and to examine the effects of the mutants on both promoter activity and the binding of UBF.

Two vertebrate rDNA promoters have been subjected to linker-scanning analysis, the human and the *X. laevis* promoters (4,6,27,28). The studies on the human promoter clearly indicated several domains important for transcription. However, several of the linker-scanning mutants examined included distance-altering mutations (4,27). The two studies on the *X. laevis* rDNA promoter actually reached very different conclusions with respect to the promoter elements (6,28 discussed in 28). Thus, a study on a third vertebrate promoter was necessary to address some of the questions raised in the initial studies. We have previously described the effects of a partial linker-scanning series on transcription *in vitro* (5), and it was necessary to determine if our *in vitro* results were reflected *in vivo*. Furthermore, to lay the groundwork for the evaluation of the interactions of the upstream and core promoter elements of the rat rDNA promoter (35) we first needed to discriminate between 'neutral' sites and sites with demonstrable, if undefined, roles in transcription. To do this a series of linker-scanning mutants (Block Substitution Mutants, BSM) of the rat rDNA promoter was constructed and the effects of these mutants on template capacity were evaluated *in vivo* and *in vitro*. Further, the behavior of these mutants, with respect to their ability to bind UBF, was analyzed in DNAse footprinting experiments.

METHODS AND MATERIALS

Templates

The templates used in this study were constructed from p5.1 (2). Some of the nucleotide substitution mutants were described previously (5). The remaining members of the BSM series were constructed essentially as described by Kunkel (29). The nucleotide sequences of these constructs, presented in Figure 1, were verified by dideoxynucleotide sequencing (30).

Transcription *In Vitro*

The conditions used for transcription *in vitro* were described previously using nuclear extract (5,31). The mutant templates were truncated at a common *Eco*R1 site to yield 638 nt transcripts and the wild-type promoter yielded a 570 nt transcript. The RNA transcribed from each promoter construct was analyzed on a 4% polyacrylamide-urea gel (2). For quantitation the gels were analyzed on an AMBIS radioanalytical analyzer.

In Vivo Assays

Chinese hamster ovary cells (CHO) were used for the analysis of the activities of the mutant promoter constructs *in vivo*. The CHO cells were maintained and cultured in DMEM supplemented with 10% fetal bovine serum (FBS). One day before transfection, 5×10^5 cells were plated on 60mm Falcon tissue culture plates. The plasmid DNAs used for transfection were purified through two rounds of CsCl ultracentrifugation. For each transfection 2 μg of plasmid DNA carrying the test promoter (Fig. 1) and 2 μg of plasmid DNA carrying a pseudo-wild type promoter were used. Transfection was performed using DEAE-dextran essentially as described (30). The pseudo wild-type gene (pseudo) is the same as the wild-type gene except for an insertion of 9 bp at +20 and therefore served as an internal control for the efficiency of transfection and for the reverse transcriptase assays.

20−24 hours after transfection, total RNA was isolated from transfected cells as described (32). 20 μg of the whole cell RNA isolated from each plate and 5×10^5 cpm of a ^{32}P-labeled primer (5'GCTGGACAAGCAAACAGCC) were used to perform primer extension assays (26,33). The products of the primer extension assays were analyzed on 6% polyacrylamide-urea sequencing gels, and quantitated as described above. Each construct was assayed at least three times.

DNase I Footprinting

End-labeled DNA probes were produced by labeling one of the two primers used to generate the PCR products to be footprinted with [γ-^{32}P] ATP and T4 polynucleotide kinase (30). The two primers used were 5'-CCATGGCCTCCTCGGTCT-3' (−208 to −191) and 5'-GGTGCAAGCCTCTTCCAACGTCC-3 (+61 to +39). The conditions used for DNase footprinting were the same as those described previously (5).

RESULTS

Characterization of the Linker-Scanning Mutants

We have previously reported that two linker-scanning mutants, BSM −129/−124 (BSM 4) and BSM 106/101 (BSM 8) demonstrated reduced responses to UBF in transcription assays *in vitro* (5). While these results were consistent with our own studies (5) that mapped binding sites for SL-1 and UBF to these regions of the UPE of the rat rDNA promoter, they did not necessarily demonstrate the physiologic significance of those two regions. Therefore, we assayed the eight linker-scanning mutants which had previously been examined *in vitro*, as well as the others depicted in Figure 1, using unfractionated nuclear extracts (Figure 2) and transiently transfected CHO cells (Figure 4).

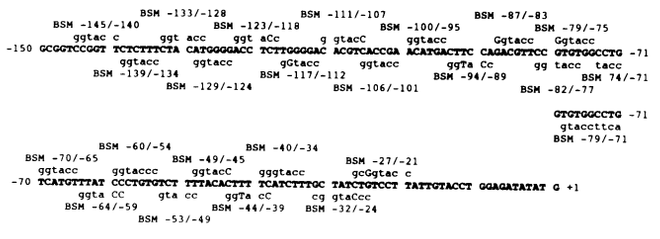

Figure 1. Schematic representation of the linker-scanning mutants of the rat rDNA promoter. The designation of each linker-scanning mutant (BSM) is indicated, as well as the bases mutated. Lower case letters indicate the bases mutated, upper case letters indicate that a base is identical to that of the wild-type promoter.

As shown in Figure 2, and presented graphically in Figure 5, several of the linker-scanning mutants supported significantly reduced levels of transcription *in vitro*: BSM −133/−128, BSM −129/−124, BSM −117/−112, BSM −106/−101, BSM −60/−54 and BSM −44/−39. The remainder of the constructs were transcribed at least 80% as well as the wild type promoter. In fact, several of the constructs were transcribed significantly better than the wild type level, e.g. BSM −100/−95 and BSM −79/−75.

In order to examine the behavior of these templates *in vivo*, a series of transfection experiments were carried out using CHO cells. As shown in Figure 3, CHO cells initiate transcription from the rat rDNA promoter at +1.

A result essentially similar to the *in vitro* transcription experiments was obtained when the linker-scanning mutants were assayed *in vivo* (Figure 4; quantitated in Figure 5). Two of the mutants, BSM −129/−124 and BSM −106/−101 were reproducibly transcribed only 40% and 50% as well as the wild-type template respectively and BSM −133/−128 and BSM −117/−112 were only 80% as active as the wild-type promoter. No other linker-scanning mutation inhibited transcription to this extent. (In any single transfection experiment there were other mutants that were not transcribed as well as the wild-type. However, after at least three independent transfection experiments, using separate plasmid preparations, they were each found to be at least 90% as efficient as the wild-type promoter.)

Several of the linker-scanning mutants were transcribed more efficiently than the wild-type promoter (Figure 5) either *in vitro* (BSM −100/−95, BSM −79/−75, and BSM −40/−33) or *in vivo* (BSM −100/−95, BSM −70/−65, BSM −53/−49, BSM −49/−55, and BSM −79/−71). BSM −100/−95 was a better template in both assays.

The properties of BSM −129/−124 and BSM −106/−101 in these *in vitro* and *in vivo* assays was consistent with our

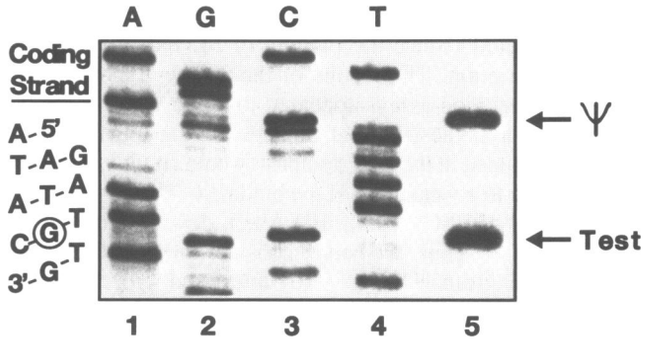

Figure 3. CHO cells initiate transcription from the rat rDNA promoter at the authentic initiation site. CHO cells were transfected as described with both the authentic rat rDNA promoter (test) and with a pseudopromoter (Ψ), which contains 9bp inserted at +20. Twenty-four hours post transfection whole cell RNA was isolated and subjected to a primer elongation assay. The products of that assay were analyzed by electrophoresis on a 6% PAGE-urea sequencing gel (lane 5) in parallel with dideoxy sequencing reactions of the promoter, using the same primer, in order to determine the initiation site (lanes 1−4). The sequence written is that of the (+) strand, the complement of the sequence determined.

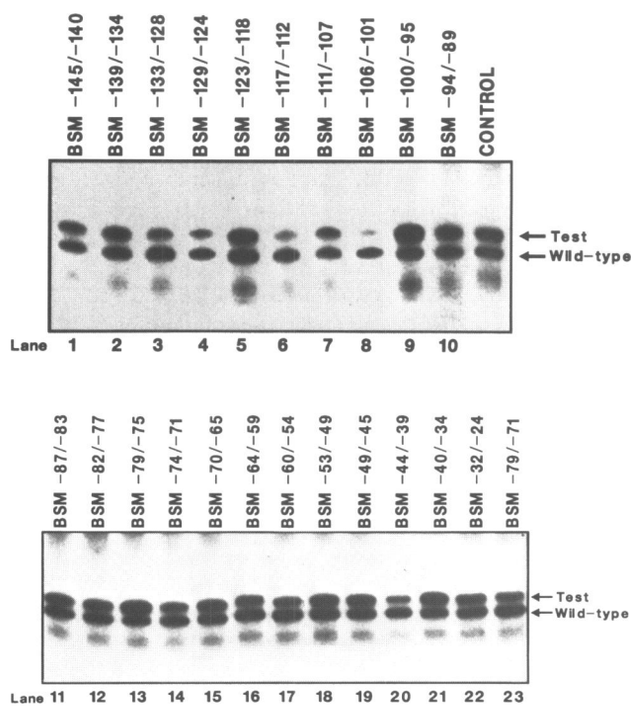

Figure 2. Linker-scanning mutants assayed *in vitro*. Equal amounts, 0.1 μg, of each linker-scanning mutant and a wild type promoter were added to a standard transcription reaction using unfractionated nuclear extract. The transcription products were resolved by denaturing PAGE, quantitated with an AMBIS radioanalytic analyzer, and autoradiographed. The upper band in each lane corresponds to the 638nt transcript of the mutant (test) promoter. The lower band corresponds to the transcript (570nt) of the wild-type promoter.

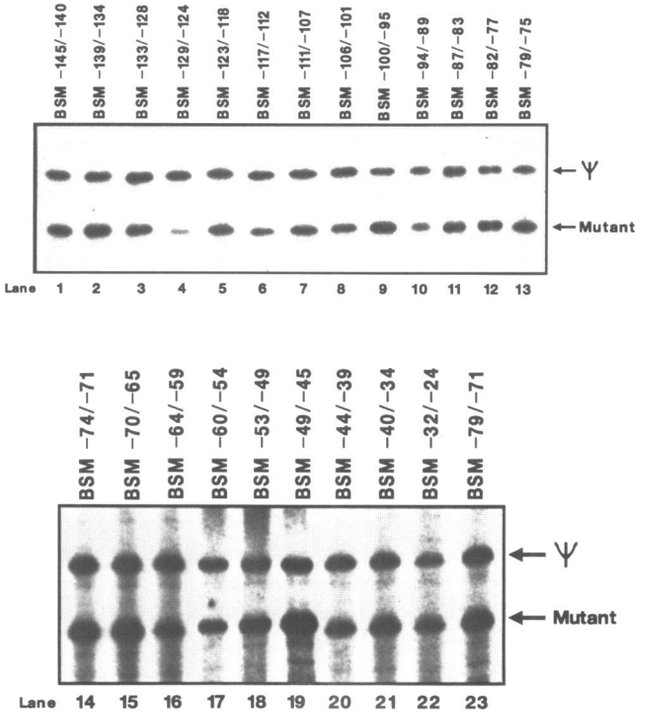

Figure 4. *In vivo* transcription of the linker-scanning mutants. CHO cells were cotransfected with the linker-scanning mutants (mutant) described in Figure 1 and the pseudo wild-type promoter (Ψ). Twenty to twenty-four hours following transfection whole cell RNA was isolated and the transcripts of the rat rDNA detected by a primer-elongation assay. The elongation products were resolved by electrophoresis on sequencing gels, quantitated with an AMBIS radioanalyzer, and autoradiographed.

previous observations that these two mutants significantly weakened the effect of the UPE on transcription from the rat rDNA promoter *in vitro* (5). We have demonstrated that (1) the footprint of UBF over the rat promoter extends from approximately −120 to −60, (2) the footprint of SL-1 is centered over −129, and (3) BSM −129/−124 bound rUBF essentially as well as the wild-type promoter in DNase footprinting assays (5).

We had hypothesized that mutating the bases between −133 and −124 would reduce the binding of SL-1 to the UPE and inhibit transcription. The results of the *in vivo* expression and *in vitro* transcription assays appeared to agree with this model. We had also hypothesized that linker-scanning mutagenesis of the region included in the UBF footprint would result in weakened promoters due to a weakining of the binding of UBF to the DNA. However, only BSM −106/−101 was a down mutant both *in vitro* and *in vivo*. Thus, we had to consider the possibilities that the nucleotides from −106 to −101 interfered with the binding of a factor other than UBF, or that they were particularly important for the binding of UBF. A series of DNAse footprinting experiments were carried out to examine these possibilities.

Analysis of the binding of UBF to the rat 45S rDNA promoter

The binding of UBF to each construct was assessed by DNase footprinting assays (Figure 6) using UBF purified through the CM-Sephadex column chromatography step (5). BSM −106/−101 significantly reduced the binding of UBF to the rat rDNA promoter on both the upper and lower strands (Figure 6, panels A and B). Although several of the mutants, e.g. BSM −100/−95, demonstrated slightly 'altered' footprints on the bottom strand in comparison to the wild type footprint (Figure 6, panel A), these mutants were clearly footprinted on the top strand (Figure 6, panel B). For example, in Figure 6, panel A a DNAse-doublet indicative of binding is a triplet in the BSM −106/−101 lane. In panel B, one of the DNAse hypersensitive sights generated when UBF binds to the promoter is present in all of the footprints lanes except when BSM −106/−101 was footprinted. The results presented clearly demonstrate that a major effect of BSM −106/−101 is to alter the protection pattern that results from the binding of UBF to the DNA. As seen in Panel B the region from −110 to −80 is less well protected than the

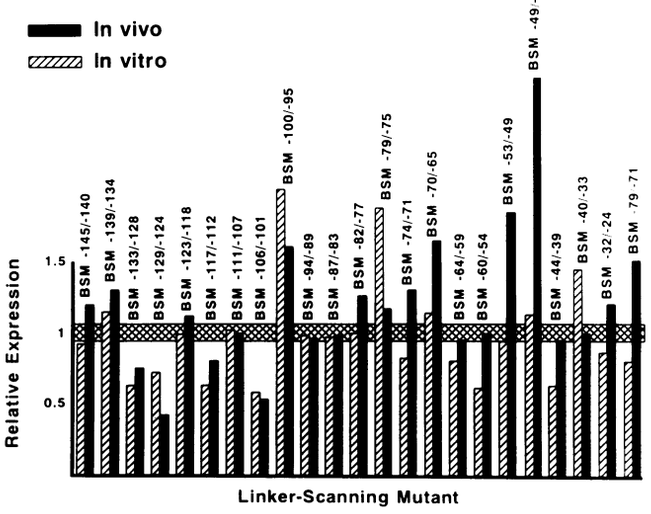

Figure 5. Graphical summary of the results of the *in vitro* and *in vivo* expression assays of the linker-scanning mutant series. The results presented for each mutant represent the average of three separate *in vitro* (□) or *in vivo* (■) transcription experiments. In each experiment the amount of transcript derived from the linker-scanning mutant was quantitated relative to the amount of transcript derived from the cotranscribed or cotransfected pseudo wild-type promoter. The stippled horizontal bar corresponds to a relative transcription of 1+/−10%.

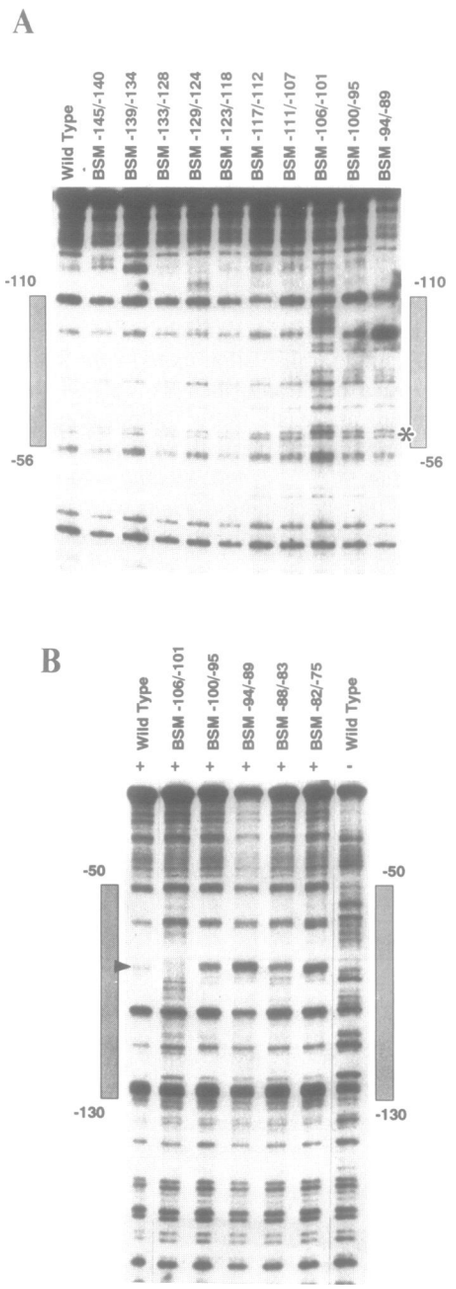

Figure 6. DNAse footprinting of the linker-scanning mutants of the rat rDNA promoter with rat UBF. Templates for footprinting were generated using the same primer pair for each mutant. The primer for either the bottom strand (A) or top strand (B) was 5'-labeled.(*) A doublet indicative of the UBF footprint on the bottom strand of the rat rDNA promoter. Note: The DNAse digestion pattern on BSM −106/−101 contains a triplet in this region. (△) a DNAse cleavage site indicative of the binding of UBF on the top strand of the rat rDNA promoter. The hatched bars alongside the autoradiographs indicate the region footprinted by UBF.

region from −80 to −55. These results suggest that for the most part, the binding of UBF to the rDNA promoter can accommodate small perturbations in the promoter sequence which, while they may affect the binding of UBF to such areas, do not affect the overall binding of UBF to the mutant promoters.

DISCUSSION

We have analyzed the effects of a series of hexanucleotide substitution mutants on transcription of the rat rDNA promoter both *in vivo* and *in vitro*. Mutations between −129 and −101 produced the most significant negative effects on transcription in both assay systems. This region is within the upstream promoter element. Two of the mutants within this 29 bp region, BSM −129/−124 and BSM −106/−101, have previously been shown to inhibit UBF-stimulated transcription by rat RNA polymerase I *in vitro* (5). On the other hand, mutating the bases from −123 to −118 (BSM −123/−118) and from −111 to −107 (BSM −111/−107) did not significantly alter the capacity of the rDNA promoter to support transcription. Thus, the results reported here are consistent with the model that the UPE consists of at least two separate but interdependent domains.

These two domains would consist of a binding site for UBF and a binding site for SL-1. The mutant BSM −106/−101 inhibits UBF-stimulated transcription, and significantly alters the UBF footprint, defining one domain within the UPE. The mutant BSM −129/−124 inhibits UBF-stimulated transcription but does not alter the binding of UBF. We hypothesize that this mutant defines a functionally important binding site for SL-1 within the UPE, as this is the site within the UPE where SL-1 footprints.

A third down mutant, BSM −117/−112, was detected both in the *in vivo* expression assays and in the *in vitro* transcription experiments with whole cell extracts, but not in our previous *in vitro* experiments using fractionated nuclear extract (5). This result is made interesting by the finding that sequences homologous to the TCTT GGGG of the rat rDNA promoter are found in widely divergent mammalian rDNA promoters in this same position (Norman Arnheim and Xiaobin Ling, personal communication). It is not clear if this site binds an additional factor, or participates somehow in the interactions between the regions defined by BSM −129/−124 and BSM −106/−101.

Several mutants, in particular, BSM −49/−45, were up mutants in the transfection experiments, but not in the *in vitro* transcription experiments. This result may reflect variations in the sensitivities of the two assay systems, due to differences in the mix of the transcription factors in the cell-free extracts versus those found *in vivo*. It may also reflect species-specific variations in the recognition of the promoter, *N.B.* the extracts are from rat cells and CHO cells were used for the transfection experiments. On the other hand, perhaps this observation should not be trivialized. Ishikawa *et al.* (36) reported that a combination of mutations of the sequence of the human rDNA promoter cerca −43 resulted in an up mutation. Thus, it may be that additional studies of this region of the mammalian rDNA promoters will be required before its function is understood.

Our results suggest that only a limited number of nucleotides within the region between −150 and −40 of the rDNA promoter are *individually* important for transcription, either *in vitro* or *in vivo*. Furthermore, despite the fact that UBF produces a large footprint over the rat promoter, only BSM −106/−101 significantly affected both transcription and the binding of UBF to the promoter. This same mutation also reduced the binding of recombinant UBF to the promoter (39). In this context it should be noted that both BSM −100/−95 and BSM −94/−89 also affect the UBF footprint slightly, but BSM −100/−95 stimulated transcription, while BSM −94/−89 had little or no effect on transcription. It may be that BSM −100/−95 actually stabilized the interaction of UBF with the promoter, in contrast to the more apparent destabilizing effect of BSM −106/−101. On the other hand, it is possible that an additional factor binds over −100/−95, within the UBF footprint.

BSM −79/−71 mutates the only significant homology found within the domains of the UBF footprints on the rat spacer and 45S promoters (34). Yet, BSM −79/−71 had no detectable affect on the binding of UBF, and no consistent affect on promoter activity *in vitro* and *in vivo*. Taken at face value, the results herein would map a very large interstitial or functionally unimportant zone between the UPE and the CPE of the rat DNA promoter. This domain would be approximately 50 bp, extending from −95 to −45. The apparent discrepancy between the transcription experiments, the results of the footprinting experiments and the UBF footprint itself led us to examine the role of this area. For this purpose a large-scale substitution mutant, BSM −95/−55, was constructed. This construct was inactive *in vivo* (data not shown), suggesting that the interstitial zone was not as large as it seemed. Apparently, the lack of an effect of the substitution mutants through this area was due to their small size (6 bp). These results may also be explained, at least in part, by the DNA-binding properties of UBF, cooperative interactions within the UPE, and cooperative interactions between the UPE and the CPE (35,37,39).

In their linker-scanning analysis of the *Xenopus laevis* rDNA promoter, Reeder *et al.* (28) found that every mutant from −142 through +1 was a down mutant to some degree. Only two of their constructs, LS −96/−87 and LS −83/−75, were more than 50% as active as the wild-type promoter *in vitro*. In contrast with the results obtained *in vitro*, LS −83/−75 was more than two-fold as active as the wild type promoter when injected into oocytes. The authors concluded that their assays 'failed to detect neutral regions between' the putative domains of the *X. laevis* rDNA promoter. On the other hand, Windle and Sollner-Webb (6) reported that between −140 and +1 of the *X. laevis* rDNA promoter there was a distinct clustering of mutants with little or no effect on transcription. This 'neutral' region stretched from −126 to approximately −69, and was flanked by domains very important to promoter activity. Interestingly, this 'neutral' region could be replaced without affecting promoter activity.

Haltiner *et al.* (4) reached a similar conclusion from their study of the effects of linker-scanning mutagenesis of the human rDNA promoter on promoter activity *in vitro*, i.e. they found a relatively neutral region that stretched from −107 to −45, that was flanked by domains more sensitive to mutagenesis. Subsequent *in vivo* studies by Haltiner-Jones *et al.* (27) essentially agreed with these observations.

Our results with hexanucleotide substitution mutants indicate that the large, central, 'neutral' region of the rat rDNA promoter is slightly smaller than that of the *Xenopus* or human promoters, extending from −94 to −45, although it is similarly placed with respect to the transcription initiation site. However, as mentioned above, the size and 'neutrality' of this region of the rat rDNA promoter is still to be defined as a construct that substituted the bases from −95 to −55 was inactive *in vivo*. Furthermore, our results do not indicate that this region is flanked by 'monolithic'

functional domains. Rather, these domains appear to consist of blocks or clusters of nucleotides of varying degrees of importance.

For example, we have recently determined that the core promoter element of the rat rDNA promoter consists of multiple domains (26), and the results reported here indicate that the UPE may also contain regions of functional significance in addition to those identified by the footprints of UBF and SL-1 (5). These observations suggest that transcription by RNA polymerase I may require the activities of more than the two already identified DNA-binding proteins, i.e. UBF and SL-1.

ACKNOWLEDGMENTS

The authors wish to express our thanks to Brian Shoop for his excellent graphic arts and Mrs. Theresa Vrona for her secretarial expertise. We wish to specifically thank Drs. Howard Morgan and Dave Carey for their comments on the manuscript. This research was supported by the Geisinger Foundation and by grants from the NIH, R01HL47638-01 and R01GM46991-01. WenQin Xie was supported by a fellowship from the American Heart Association, Western Pennsylvania Affiliate.

REFERENCES

1. Mandal, R. (1984) *Prog. Nucl. Acids Res. Mol. Biol.* **31**, 115–160.
2. Rothblum, L., Reddy, R. and Cassidy, B. (1982) *Nucl. Acids Res.* **10**, 7345–7362.
3. Sollner-Webb, B. and Tower, J.A. (1986) *Ann. Rev. Biochem.* **55**, 801–830.
4. Haltiner, M., Smale, S. and Tjian, R. (1986) *Mol. Cell. Biol.* **6**, 227–235.
5. Smith, S., Oriahi, E., Yang-Yen, H.-F., O'Mahony, D., Rose, K., Chen, K. and Rothblum, L. (1990) *Molec. Cell. Biol.* **10**, 3105–3116.
6. Windle, J., and Sollner-Webb, B. (1986) *Mol. Cell. Biol.* **6**, 4585–4593.
7. Henderson, S., and Sollner-Webb, B. (1990) *Mol. Cell. Biol.* **10**, 4970–4973.
8. Grummt, I., Kuhn, A., Bartsch, I. and Rosenbauer, H. (1986) *Cell* **47**, 901–911.
9. Henderson, S., and Sollner-Webb, B. (1986) *Cell* **47**, 891–900.
10. McStay, B. and Reeder, R.H. (1986) *Cell* **47**, 913–920.
11. Bell, S., Jantzen, H.-M. and Tjian, R. (1990) *Genes Dev.* **4**, 943–954.
12. Cassidy, B., Haglund, R. and Rothblum, R. (1987) Biochim. Biophys. Acta. **909**, 133–144.
13. Tower, J., Culotta, B. and Sollner-Webb, B. (1986) *Mol. Cell. Biol.* **6**, 3451–3462.
14. McStay, B. and Reeder, R. (1990) *Genes Dev.* **4**, 1240–1252.
15. Bateman, E. and Paule, M. (1988) *Cell* **54**, 985–992.
16. Henderson, S. and Sollner-Webb, B. (1989) *Genes Dev.* **3**, 212–223.
17. Bell, S., Learned, R., Jantzen, H.-M. and Tjian, R. (1988) *Science* **241**, 1192–1197.
18. Learned, R., Learned, T., Haltiner, M. and Tjian, R. (1986) *Cell* **45**, 847–857.
19. Learned, R., Cordes, S., Tjian, R. (1985) *Mol. Cell. Biol.* **5**, 1358–1369.
20. Miesfeld, R. and Arnheim, N. (1984) *Mol. Cell. Biol.* **4**, 221–227.
21. Mishima. Y., Financsek, L., Kominami, R. and Muramatsu, M. (1982) *Nucl. Acids Res.* **10**, 6659–6670.
22. Pikaard, C.S., Smith, S.D., Reeder, R.H. and Rothblum, L. (1990) *Mol. Cell. Biol.* **10**, 3810–3812.
23. Jantzen, H.-M., Admon, A., Bell, S. and Tjian, R. (1990) *Nature* (London) **344**, 830–835.
24. O'Mahony, D., and Rothblum, L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3180–3184.
25. O'Mahony, D.J., Xie, W.Q., Smith, S.D., Singer, H.A. and Rothblum, L.I. (1992) *J. Biol. Chem*, **267(1)**, 35–38.
26. Xie, W.-Q., O'Mahony, D., Smith, D. and Rothblum, L. (1991) *Mol. Cell. Biochem.* **104**, 127–135.
27. Haltiner-Jones, M., Learned, R. and Tjian, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 669–673.
28. Reeder, R., Pennock, D., McStay, B., Roan, J., Tolentinto, E. and Walker, P. (1987) *Nucl. Acids Res.* **15**, 7429–7441.
29. Kunkel, T. (1985) *Prod. Natl. Acad. Sci. USA* **82**, 488–492.
30. Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. and Struhl, K. (1987) Current Protocols in Molecular Biology. Wiley Interscience, New York.
31. Haglund, R.E. and Rothblum, L.I. (1987) *Mol. Cell. Biochem.* **73**, 11–25.
32. Xie, W.-Q. and Rothblum, L. (1991) *Bio. Tech.* **11(3)**, 324–329.
33. Zehring, W. and Greenleaf, A. (1990) *J. Biol. Chem.* **259**, 8351–8353.
34. Smith, S., Oriahi, E., Yang-Yen, H.-F., Xie, W.-Q., Chen, C. and Rothblum, L. (1990) *Nucl. Acids. Res.* **19**, 1677–1685.
35. Xie, W.-Q. and Rothblum, L. (1992) *Mol. Cell. Biol.*, in press.
36. Ishikawa, Y., Safrany, G., Hisatake, K., Tanaka, N., Maeda, Y., Kato, Y.H., Kominami, R. and Muramatsu, M. (1991) *J. Mol. Biol.* **218**, 55–67.
37. McStay, B., Frazier, M.W. and Reeder, R.H. (1991) *Genes and Dev.* **5**, 1957–1968.
38. Cassidy, B., Yang-Yen, H.-F. and Rothblum, L. (1987) *Mol. Cell. Biol.* **7**, 2388–2396.
39. O'Mahony, D.J., Smith, S.D., Xie, W.Q. and Rothblum, L.I. (1992) *Nucl. Acids Res.*, in press.