

Enhancers for RNA Polymerase I in Mouse Ribosomal DNA

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The intergenic spacer of the mouse ribosomal genes contains repetitive 140-base-pair (bp) elements which we show are enhancers for RNA polymerase I transcription analogous to the 60/81-bp repetitive enhancers (enhancers containing a 60-bp and an 81-bp element) previously characterized from *Xenopus laevis*. In rodent cell transfection assays, the 140-bp repeats stimulated an adjacent mouse polymerase I promoter when located in *cis* and competed with it when located in *trans*. Remarkably, in frog oocyte injection assays, the 140-bp repeats enhanced a frog ribosomal gene promoter as strongly as did the homologous 60/81-bp repeats. Mouse 140-bp repeats also competed against frog promoters in *trans*. The 140-bp repeats bound UBF, a DNA-binding protein we have purified from mouse extracts that is the mouse homolog of polymerase I transcription factors previously isolated from frogs and humans. The DNA-binding properties of UBF are conserved from the mouse to the frog. The same regulatory elements (terminators, gene and spacer promoters, and enhancers) have now been identified in both a mammalian and an amphibian spacer, and they are found in the same relative order. Therefore, this arrangement of elements probably is widespread in nature and has important functional consequences.

The genes coding for the large rRNAs of most eucaryotes are organized in a similar fashion. From yeast cells to humans, these genes are arranged in multiple tandem copies with precursor-coding regions separated from each other by intergenic spacers (reviewed in references 42 and 54). Recent work from a number of laboratories has suggested that, at least among the multicellular eucaryotes, there is also a broadly conserved arrangement of transcriptional regulatory elements in the spacer (reviewed in reference 49a). The ribosomal genes of the frog, *Xenopus laevis*, may be considered a paradigm for this type of organization since all of the known regulatory elements have been identified in this organism.

A typical intergenic spacer from an *X. laevis* ribosomal gene is shown in the top line of Fig. 1, with the spacer from a mouse ribosomal gene shown below for comparison. In *X. laevis*, the intergenic spacer is bounded on the left by a site for 3'-end formation of the precursor (31) and on the right by the gene promoter that directs initiation of the precursor transcript. Between these points are located one or more spacer promoters (4, 41, 53) (the only other known promoters that are recognized by polymerase I), and downstream of the spacer promoters are repetitive 60- and 81-base-pair (bp) elements (60/81-bp elements) that act as enhancers for polymerase I transcription and are additive in effect (11, 30, 44, 48). Between the enhancers and the gene promoter is a termination site (31, 40). The 60/81-bp enhancers bind a *Xenopus* transcription factor, xUBF, which also binds to the gene promoter (47) and is the frog homolog of human UBF (hUBF; 2) and rat UBF (rUBF; 48a), factors which also stimulate transcription from the gene promoters of these species. The frog activity TFIS (15), which has similar properties, almost certainly contains xUBF.

We particularly note the similarity in arrangement be-

tween elements of the frog and mouse intergenic spacers, despite the fact that these two spacers differ in length by almost an order of magnitude and show no obvious sequence homology. As does the frog spacer, the mouse spacer has sites for termination of the precursor transcript (23), a spacer promoter (29, 56), a promoter-proximal terminator (22, 25), and a gene promoter. The only discordant note in the frog-mouse comparison is that, until now, no elements that function as do the frog enhancers have been identified in a mammal. If we accept the frog model as a guide, we would predict that such enhancer elements would exist in mouse ribosomal genes between the spacer promoter and the gene promoter and would consist of repeated elements. It has been known for some time that there is a 140-bp repeated sequence in the mouse spacer that is present in this particular location (28). In this paper, we show that these 140-bp repeated elements are mouse ribosomal gene enhancers, analogous in function to the 60/81-bp enhancer elements in *X. laevis*, and that their action is highly conserved in evolution.

MATERIALS AND METHODS

Plasmids. (i) **Mouse rDNA constructs.** p5'-230 contains the mouse ribosomal DNA (rDNA) region from -230 to +292 (containing the complete gene promoter) inserted between the *EcoRI* and *PvuII* sites of pBR322 (25). p5'-1800 contains ribosomal sequences from ~-1800 to +292 between the same vector sites (or, for the plasmid used for Fig. 2C, between the *SalI* and *PvuII* sites of pBR322). p5'-1800 includes the rDNA sequences of p5'-230 plus the adjoining upstream *SalI* fragment containing 11 and 2/3 copies of the ~140-bp repeats. p140 contains 11 and 2/3 repeats of mouse enhancer elements (from residue -1800 to -168) that have been S1 blunted and inserted into the *SmaI* site of pUC18. The ends of the rDNA insert of p140 retain less than half of the residues of a functional terminator sequence. pT36

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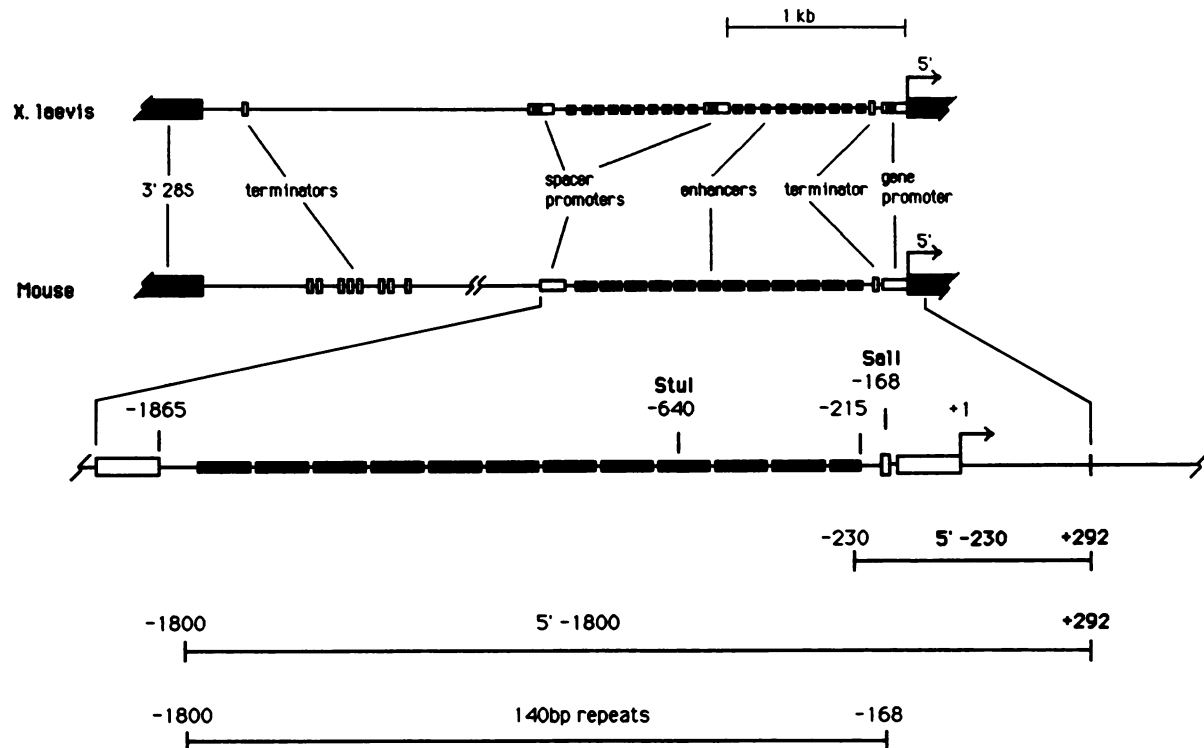


FIG. 1. Comparison of intergenic spacer regions of frog and mouse rDNAs. At the top is shown the intergenic spacer from a typical repeating unit of *X. laevis* rDNA. The locations of various transcriptional regulatory elements are indicated. The small black boxes indicate the 42-bp sequence element that is present in each enhancer as well as in each promoter. In *X. laevis*, the terminator-like element downstream of the 28S coding region actually contains a natural point mutation that retains the ability to form 3' ends but allows polymerase to read through into the spacer (32). In the diagram of the mouse intergenic spacer, about 20 kilobases of sequence, of unknown function, has been deleted between the slash marks. The small black boxes in this case represent the 140-bp repetitive enhancer elements. The parent clone of the mouse spacer used in this study contains 11 and 2/3 copies of the 140-bp repeats between positions -1770 and -215 . kb, Kilobase.

contains a 36-bp *Hpa*II fragment that includes the functional mouse polymerase I terminator which surrounds the *Sal*I site at position -168 of the ribosomal DNA. This fragment was cloned in pUC18 (pT38; 56). The position of each of these rDNA regions within the intact repeating unit is shown in Fig. 1.

(ii) *X. laevis* rDNA constructs. The A gene plasmid contains rDNA residues -245 to $+13$ upstream of a prokaryotic tester segment inserted between the *Sal*I and *Hind*III sites of pUC18. The E plasmid contains a block of 10 *X. laevis* 60/81-bp enhancers (residues -965 to -245) in the *Sma*I site of pSP65. The *X. laevis* constructs used for Fig. 5 and 7 are described in references 44 and 30, respectively. In addition, a block of mouse 140-bp repeats (shown in Fig. 1) was attached to an *X. laevis* promoter at position -245 .

Transcription analysis in the mouse. S-100 extract was prepared from mouse L1210 cells, and *in vitro* transcription assays were performed as described elsewhere (26, 55), with DNA amounts and incubation times as indicated in the figure legends. Transfection assays were performed in CHO cells by using the DEAE dextran-dimethyl sulfoxide shock protocol as described previously (25). The *trans* competition experiment shown in Fig. 2C utilized $\sim 5 \times 10^5$ cells, 2 μ g of template plasmid per ml, and 5 μ g of the competitor plasmid per ml per 60-mm dish; the *cis* stimulation assay shown in Fig. 3 used $\sim 10^6$ cells and 3 μ g of the 5'-1800 template per ml (or an equimolar amount of the 5'-230 template, brought up to 3 μ g/ml by the addition of pUC8 DNA) per 60-mm dish. Both the *in vitro* and *in vivo* transcripts (the latter were isolated 24 h posttransfection) were analyzed by S1 nuclease

protection using a single-stranded probe, 5' end labeled at residue $+155$ or $+292$ as described in reference 25.

Transcription analysis in *Xenopus* oocytes. Plasmids were injected into oocytes from *X. laevis* (Fig. 7A) or *Xenopus borealis* (Fig. 6 and 7B) (52), both of which have been traditionally utilized to assess *Xenopus* rDNA transcription, and transcripts were analyzed by S1 nuclease protection using single-stranded 5'-end-labeled probes. Current versions of these techniques are described fully in references 31 and 44. For the *trans* competitions shown in Fig. 6, the oocytes were injected with a mixture of 0.75 ng of template plasmid (the A gene) and 0.75 ng of competitor plasmid. For the experiment of Fig. 7A, the oocytes were coinjected with a 0.1 fmol of each of the indicated plasmids (making a total of 0.5 ng of DNA in the injection of lane 1). For the *cis* stimulations of Fig. 6B, oocytes were injected with 0.4 fmol of the indicated template, the smaller plasmids being supplemented with pUC18 DNA to achieve a constant 1.5 ng of total injected DNA.

Purification of mUBF. Mouse UBF (mUBF) was purified by passage over DEAE Sephacel (0.12 to 0.26 M KCl fraction), phosphocellulose (0.6 to 0.8 M KCl fraction), Mono Q (0.45 to 0.47 M KCl fraction, based on the fast protein liquid chromatography theoretical line), and a frog enhancer oligoaffinity column (0.35 to 1.0 M KCl fraction; 47). After the first step, activity was monitored by footprinting. Renaturation of UBF was performed as described in reference 47.

DNase I footprinting. DNase I footprinting was performed on end-labeled probes essentially as described in reference

19. Affinity-purified xUBF was prepared as described in reference 47. Footprinting probes were for *X. laevis* promoter, the *Sall* (–245)-to-*Bam*HI (+49) fragment of pseudo 40 labeled at the *Bam*HI site; for *X. laevis* enhancers, a 140-bp *Pst*I fragment of the enhancers subcloned into Bluescript and labeled at the *Eco*RI site of the polylinker; for mouse promoter, the *Sall* (–168)-to-*Xma*I (+155) fragment labeled at the *Xma*I site; for mouse enhancers, the *Stu*I (–640)-to-*Sall* (–168) fragment labeled at the *Stu*I site.

RESULTS

Mouse 140-bp repeats have both negative (*trans*) and positive (*cis*) effects on the mouse ribosomal gene promoter. A number of different assays have been employed to demonstrate that the 60/81-bp enhancer repeats of the *X. laevis* intergenic spacer influence transcription. The most commonly used assay has been one in which two promoters are coinjected into the nuclei of frog oocytes, with one promoter bearing enhancers and the other not. In this competition assay, the transcriptional balance shifts in favor of the promoter bearing the enhancers in *cis* (10, 51). This result incorporates two separable effects of the enhancers: stimulation of a promoter in *cis* and competition against a promoter in *trans*. The *trans* competition effect of the *Xenopus* enhancers can be directly demonstrated by placing subcloned enhancers in competition against a promoter on a separate plasmid, either in microinjected oocytes (30) or in vitro (44, 47). More recently, the enhancer elements have been shown to stimulate a promoter in *cis* in singly injected oocytes (an assay akin to the transient expression assays normally used to detect enhancers of polymerase II promoters) and in oocyte nuclear homogenates (44). However, the 60/81-bp repeats do not show *cis* stimulation in whole-cell, S-100-type extracts in vitro (L. Pape, unpublished observations; B. McStay, unpublished observations). This lack of *cis* stimulation in such in vitro extracts is also characteristic of most polymerase II enhancers.

Because of their repetitive nature and their location within the intergenic spacer, we suspected that the mouse 140-bp repeats could be mammalian enhancers for polymerase I. We therefore tested their activity in both the *cis* and the *trans* assays mentioned above. In preliminary experiments we found that mouse promoters attached to the 140-bp repeats were transcribed no better than promoters lacking the repeats when assayed in vitro in mouse S-100 transcription extracts (data not shown). This absence of a *cis* effect in vitro is consistent with results of similar experiments done in S-100 extracts with the prototype *Xenopus* enhancer. The 140-bp repeats did, however, have a negative effect in vitro when present in *trans* to a mouse promoter (Fig. 2). Preincubation of the mouse extract with the subcloned 140-bp repeats decreased transcription from a subsequently added mouse promoter up to 10-fold, while an equal mass of plasmid vector had no effect. The same inhibition was observed whether or not the test promoter had 140-bp repeats attached in *cis* (compare Fig. 2A with B). Such *trans* competition was also seen if the 140-bp repeats were added to the reaction at the same time as the promoter (data not shown). These results suggest that the 140-bp repeats are competing for the binding of a transcription factor which is essential for the functioning of the gene promoter.

trans competition by the mouse 140-bp repeats could also be observed in transient expression assays in which rodent cells were cotransfected with separate plasmids. In the experiment of Fig. 2C, cells were cotransfected with one

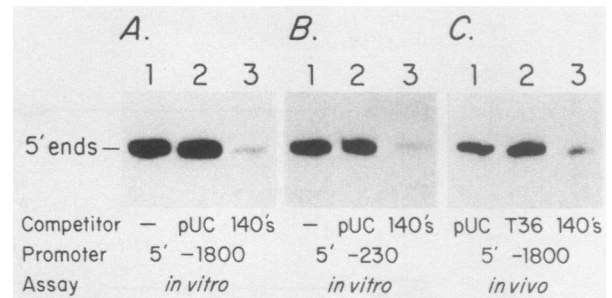


FIG. 2. *trans* competition of mouse ribosomal gene promoter by mouse 140-bp repeats. (A) Analysis in vitro. S-100 transcription reactions were preincubated with no DNA (lane 1), with 150 ng of pUC18 vector DNA (lane 2), or with 150 ng of pUC18 carrying the block of 140-bp repeats (lane 3; fragment from –1800 to –168, shown in Fig. 1). After 60 min, closed circular 5'-1800 template was added (200 ng to lane 1 and 50 ng each to lanes 2 and 3). Five minutes later, a 30-min transcription reaction was initiated by the addition of ribonucleoside triphosphates. RNA was then extracted and analyzed by S1 nuclease protection. (B) Analysis in vitro. Same as in panel A except that the template was 5'-230. (C) Analysis in vivo. CHO cells were transiently transfected with 5'-1800 and a 2.5-fold excess of the following plasmids: pUC18 (lane 1); T36 (lane 2; T36 is a 36-bp mouse promoter-proximal terminator element subcloned in pUC18); the block of 140-bp repeats subcloned in pUC18 (lane 3). Transcription was analyzed by S1 nuclease protection. Similar results were obtained with the 5'-230 template (data not shown).

plasmid carrying a mouse promoter and a second plasmid either lacking or carrying the subcloned block of 140-bp elements (Fig. 2C, lanes 1 and 3). The observed *trans* competition by the mouse 140-bp repeats was approximately equally efficient in vivo and in vitro and was comparable to that exerted by the *Xenopus* 60/81-bp enhancer repeats in *trans* in frog oocyte assays.

One potential concern with the experiment of Fig. 2 is that the end of the subcloned 140-bp repeat fragment contains a truncated partial polymerase I terminator sequence (see Materials and Methods), for it is known that complete terminator elements can influence the activity of a gene promoter (11, 25, 26, 36). However, we have shown that these partial sequences do not exhibit terminator activity (25, 26; unpublished observations). Furthermore, a complete subcloned mouse terminator did not compete against the promoter in this assay (Fig. 2C, lane 2), and a subcloned derivative of the 140-bp repeat fragment that completely lacks these partial terminator sequences was also an efficient competitor of a promoter in *trans* (data not shown). Therefore, we conclude that the *trans* competition seen in Fig. 2 is due to the 140-bp repeats themselves.

We next tested the ability of the 140-bp repeats to show *cis* stimulation of a mouse ribosomal gene promoter in vivo (Fig. 3). When an rRNA gene promoter linked to the 140-bp repeats (construct 5'-1800 [Fig. 1]) was singly introduced into rodent cells by transient transfection, it directed considerably higher levels of transcription than did an otherwise identical plasmid that lacked the repeat elements (construct 5'-230 [Fig. 1]) tested in a parallel transfection. This result has been reproducibly obtained in eight separate experiments. In many of these experiments, the transfection solution also contained as an internal control a 5S gene (44) which yielded virtually identical ($\pm 15\%$) amounts of transcript in the different cell cultures. Thus, the mouse 140-bp repeats stimulate in vivo transcription when located in *cis* to a mouse ribosomal gene promoter. This level of stimulation



FIG. 3. *cis* stimulation of the mouse ribosomal gene promoter by mouse 140-bp repeats. Equimolar amounts of plasmids 5'-230 (lane 1) and 5'-1800 (lane 2) were transfected into cultured CHO cells, and transcripts were analyzed by S1 nuclease protection. For the transfection shown in lane 1, the total DNA concentration was made to equal that of lane 2 by the addition of pUC18 cloning vector.

by the mouse 140-bp repeats is similar to that observed for the *Xenopus* 60/81-bp enhancer repeats in analogous assays with *Xenopus* systems (44).

Purification of mUBF. The observation that the mouse 140-bp repeats compete against the mouse promoter (Fig. 2) suggests that both the promoter and the repeat elements bind one or more common transcription factors. In *X. laevis*, it has been shown that a polymerase I transcription factor, xUBF, binds to the 60/81-bp enhancer elements as well as to the gene promoter and that its binding is intimately related to the activity of the 60/81-bp elements (47). We thus hypothesized that a similar UBF factor is present in the mouse and acts on mouse 140-bp enhancerlike repeats.

DNase I footprinting using extracts from mouse cells revealed an activity that specifically bound to the mouse 140-bp repeats (Fig. 4A) and caused distinctive footprints which mimicked those of xUBF (Fig. 5). We purified this mUBF to homogeneity, using its distinctive footprint on the mouse 140-bp repeats as the assay. The purification involved passage over DEAE-cellulose, phosphocellulose, Mono Q, and oligonucleotide affinity resins (see Materials and Methods). Electrophoretic resolution of the resultant polypeptides revealed two closely spaced bands of 97- and 94-kilodalton apparent molecular weights (Fig. 4B), the same size as hUBF and rUBF polypeptides (2, 48a). By excising the 97- and 94-kilodalton bands from a sodium dodecyl sulfate-polyacrylamide gel and renaturing the protein, the mouse footprinting activity was shown to coelectrophorese with these two polypeptides (Fig. 4C). Since this mouse component has the same polypeptide profile (Fig. 4B) and the same footprinting properties (Fig. 5) as other mammalian UBF preparations, we identify it as mUBF.

140-bp repeats bind UBF from both mouse and frog. The footprinting characteristics of purified mUBF and xUBF are shown in Fig. 5. As noted above, mUBF produced a distinctive footprint on the mouse 140-bp repeats (Fig. 4A and 5A). Strikingly, xUBF produced a virtually identical footprint (Fig. 5A). mUBF and xUBF also produced virtually identical footprints on the *X. laevis* 60/81-bp enhancers (Fig. 5B). As is characteristic of xUBF on the *Xenopus* enhancer (47), the UBF proteins of both species decreased DNase I accessibility throughout the entire region of the mouse enhancer except at a small number of positions at which they caused increased cleavage (Fig. 4A). Furthermore, mUBF also produced footprints on the *X. laevis* ribosomal gene promoter (Fig. 5C), the mouse ribosomal gene promoter (Fig. 5D), and the human ribosomal gene promoter (not shown) that are extremely similar to those produced by xUBF. It is noteworthy that UBF caused footprints throughout the promoter domains in mice (this work), humans (3, 34), and *Xenopus* species (47), which is

consistent with the idea that UBF-binding domains are important determinants of polymerase I promoter architecture. Although on the mouse ribosomal gene promoter the UBF footprints are reproducible and specific (Fig. 5D), when less-purified UBF preparations are used the footprint is not as pronounced as those on the enhancers or the *Xenopus* promoter, perhaps explaining why footprints were not detected on the mouse rDNA promoter in previous attempts (34). From the DNA-binding studies we conclude that UBF binds to the mouse 140-bp repeats, the *X. laevis* enhancers, and the mouse and frog ribosomal gene promoters. Because their DNA-binding properties are essentially identical, we further conclude that mUBF and xUBF are homologs, a conclusion which has also been reached by comparison of xUBF with UBF purified from humans and rats.

Since the frog enhancer- and promoter-binding factor xUBF bound to the mouse 140-bp repeats (Fig. 5), we would expect the 140-bp elements to compete in *trans* against a frog promoter in a frog transcription system, as do the *Xenopus* repeats which bind xUBF. Figure 6 shows that this prediction was validated in *Xenopus* oocyte coinjection experiments. Notably, the same extent of competition was seen whether the *Xenopus* promoter was coinjected with the mouse 140-bp repeats (Fig. 5, lane 3) or with the *Xenopus* enhancer repeats (lane 2). Thus, the mouse 140-bp repeats efficiently compete against a heterologous frog promoter in *trans*, presumably by competing for the same factor as the frog enhancers.

Mouse 140-bp repeats enhance heterologous frog promoters in oocyte injection assays. So far we have shown that the mouse 140-bp repeats mimicked the frog 60/81-bp enhancers in three important aspects: (i) they competed against both frog and mouse promoters in *trans* (Fig. 2 and 6); (ii) they stimulated their homologous promoter in *cis* (Fig. 3); and (iii) they bound mUBF and xUBF, factors which also bind to frog and mouse promoters (Fig. 5). In Fig. 7, we show the striking result that the mouse 140-bp repeats enhanced a heterologous frog promoter when attached to that promoter in *cis*. For these experiments the block of mouse 140-bp repeats was ligated, in both orientations, onto a frog ribosomal gene promoter at position -245, approximately the same position at which the endogenous frog enhancers are normally attached. Each enhancer-bearing construct was then injected into frog oocytes along with an equimolar amount of a frog promoter lacking enhancer repeats, and the RNA derived from these two differently marked constructs is shown in the first and second tracks, respectively, of each set of lanes in Fig. 7A. The presence of a block of mouse 140-bp repeats gave the attached frog promoter a transcriptional advantage over a promoter lacking the repeats, for the experimental promoter directed production of considerably more RNA than did the coinjected control promoter (lanes 3 versus lanes 1). In different experiments this advantage varied between 5- and 20-fold. In multiple injections we observed a similar degree of stimulation regardless of the orientation of the 140-bp repeats (for example, see Fig. 7A, lanes 3 and 4). Twelve 140-bp repeats reproducibly have about the same effect as 10 homologous 60/81-bp repeats from the frog (compare lane 2 with lanes 3 and 4). This suggests that the heterologous elements are approximately as active as the homologous elements in stimulating transcription in this assay.

Mouse 140-bp repeats also stimulated a frog promoter in *cis* in the absence of a competing promoter (Fig. 7B). Again, the positive effect was seen when the repeats were in either

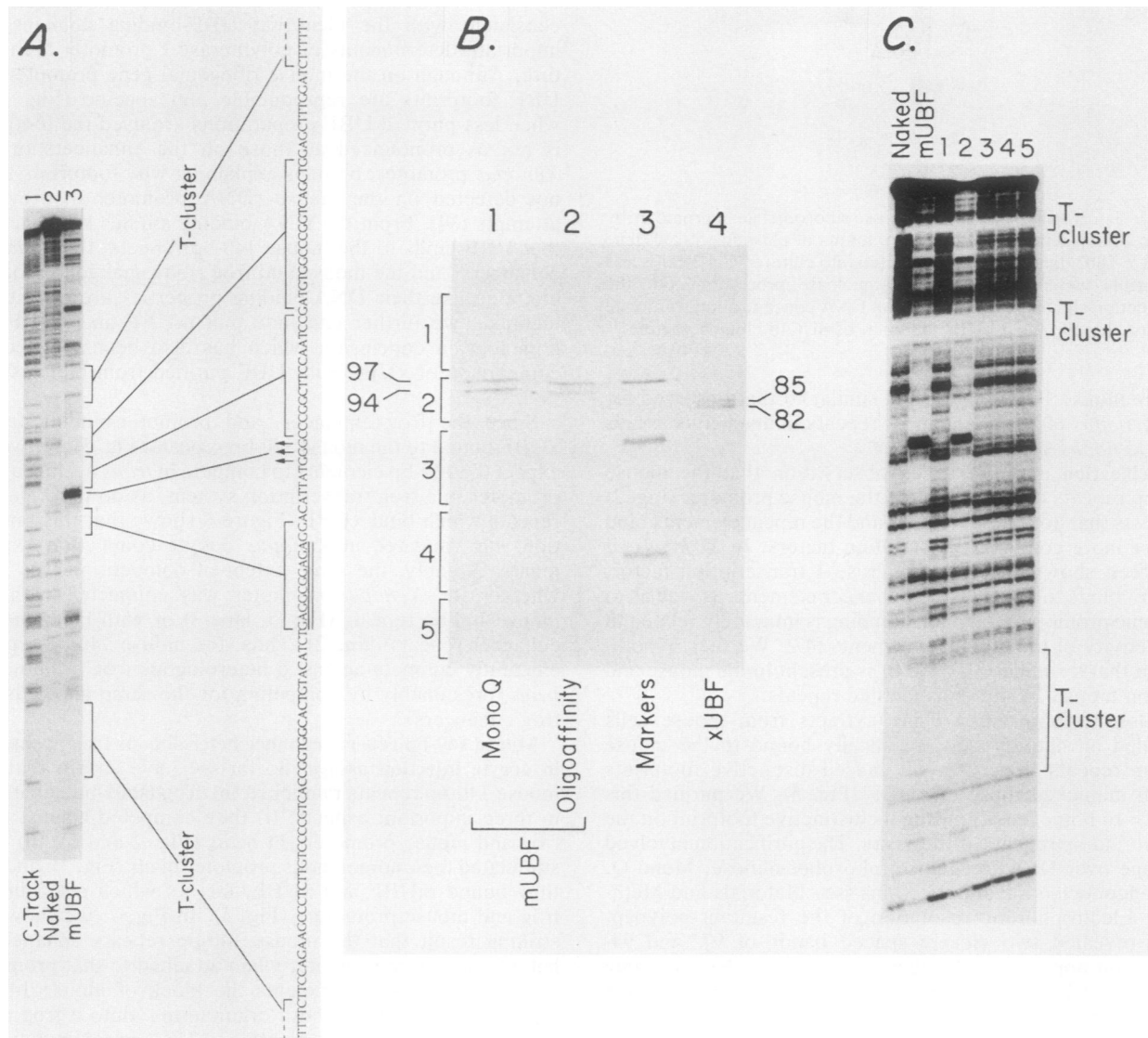


FIG. 4. Purification of mUBF. (A) Footprinting of mUBF on mouse 140-bp repeats. The footprint of affinity-purified mUBF on the 140-bp element is shown (lane 3) aligned relative to the digestion pattern of the naked DNA (lane 2) and to a C track from a chemical sequence analysis of the DNA (lane 1). The end-labeled DNA used for footprinting was the same as that used in Fig. 5A. Each 140-bp element is bounded by clusters of T residues. mUBF caused a cluster of three strong hypersensitive sites (indicated by arrows) approximately in the middle of each repeat, with footprint protection (indicated by lines above the sequence) on each side of the hypersensitive sites. The footprint to the 5' side of the hypersensitive site coincides with a sequence that is conserved in spacer repeats from mice, rats, and Chinese hamsters (underlined sequence). A related element, GAGCCCGG, is present in the spacer of human ribosomal genes (14). (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of mUBF and xUBF. Extracts prepared from mouse cells were fractionated sequentially on DEAE-cellulose, phosphocellulose, Mono Q and a DNA affinity column containing the frog enhancer sequences, as described in Materials and Methods. Lane 1, mUBF after Mono Q chromatography; lane 2, mUBF after DNA affinity chromatography; lane 3, molecular size markers of 97.4, 68, and 43 kilodaltons; lane 4, affinity-purified xUBF. The gel was silver stained. (C) Renaturation of mUBF from a sodium dodecyl sulfate-polyacrylamide gel. Protein (~1 µg) from the Mono Q step (about 50% mUBF) was electrophoresed on sodium dodecyl sulfate-polyacrylamide gel, and the gel was cut into five slices as indicated to the left of the gel in panel A. Protein was eluted from each slice, renatured, and used for DNase I footprinting on the mouse 140-bp repeats (the same probe used in Fig. 5A). Lanes 1 to 5, Footprints obtained with the protein eluted from gel slices 1 through 5 (B), respectively, compared with the DNase digestion pattern of the naked probe or with 5 ng of affinity-purified mUBF. This particular experiment shows a slight gel compression in the bands in the +40 region (outside of the region that shows UBF footprinting).

orientation, and the degree of stimulation was similar to that seen with the homologous 60/81-bp elements. We conclude that in all of the assays available to us, the mouse 140-bp repeats behaved as true *cis* stimulatory enhancers. Furthermore, their ability to function in a heterologous system indicates that this enhancer function is widely conserved in evolution.

DISCUSSION

Mouse 140-bp repeats are enhancers for polymerase I transcription analogous to the *X. laevis* 60/81-bp enhancers. The ability of spacer sequences to affect polymerase I transcription was first demonstrated for *X. laevis* in experiments involving the addition or deletion of large portions of

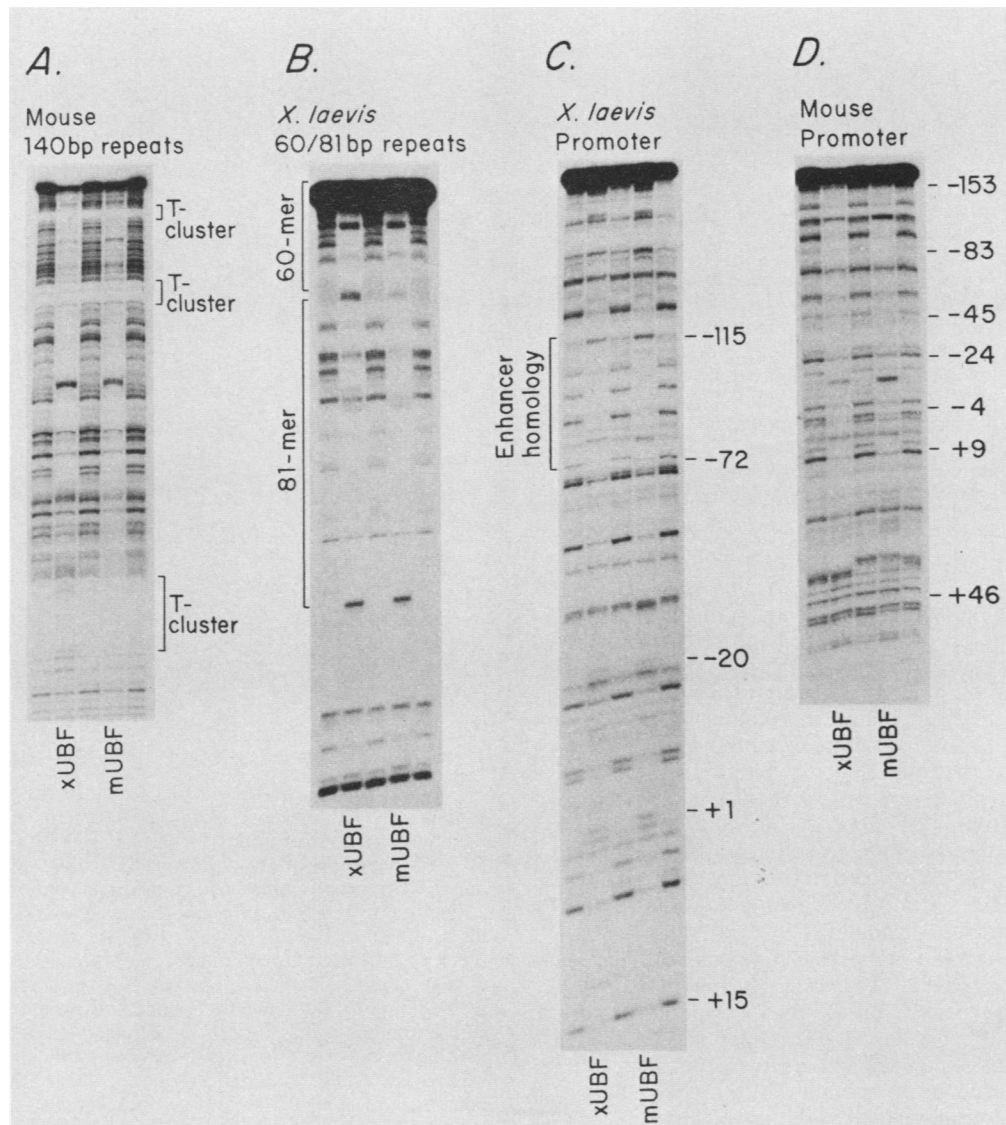


FIG. 5. DNase I footprinting of mouse and frog UBFs. Each end-labeled footprinting probe was mixed with affinity-purified xUBF or mUBF and digested with DNase I, and the products were electrophoresed on a polyacrylamide gel. Note that in all cases the alterations in DNase I digestion caused by xUBF are closely matched by those caused by mUBF. (A) Mouse 140-bp repeats. Three repeats are shown, each consisting of a 115-bp element plus a poly(T) cluster. (B) *X. laevis* 60/81-bp repeats. An 81- and a 60-bp element are shown. (C) *X. laevis* promoter. Alterations in DNase I digestion are most visible over the upstream region of the promoter (including the region with homology to the enhancers, residues -115 to -72) as well as around the initiation site (predominantly residues -20 to +15). (D) Mouse promoter. UBF causes reproducible footprints over much of the promoter, including the upstream region from -150 to about -75 as well as the region surrounding the initiation site (residues -20 to +9).

the spacer (5, 40, 51). Subsequent finer dissection showed that a large part of the stimulatory activity resides in the blocks of repetitive 60/81-bp enhancer elements (11, 30, 44, 48). A list of the distinguishing characteristics of the 60/81-bp elements would include the following. (i) These elements reside between a spacer promoter and the gene promoter. (ii) They are present in multiple copies at a genetically unstable number. (iii) They bind a factor in common with the gene promoter (47) and compete against a gene promoter located in *trans*. (iv) They stimulate a promoter in *cis* in the presence (30) or absence (44) of a competitor template. (v) *cis* enhancement is observed in *vivo* but not with S-100-type in *vitro* systems. However, *trans* competition is seen both in *vivo* (30) and in *vitro* (44, 47). (vi) The *cis*-effect is relatively

orientation independent, occurs over significant distances (30, 44), and can be observed even when a promoter-bearing plasmid is concatenated with an enhancer-bearing plasmid (16). (vii) Their effect is proportional to the number of copies that are present (11, 44, 48). (viii) They affect only specific transcription by RNA polymerase I (44; P. Labhart, unpublished data).

The *X. laevis* 60/81-bp repeats have been called enhancers by analogy with the RNA polymerase II regulatory elements that are able to function in both orientations and over large distances. The fact that the 60/81-bp enhancers have a 42-bp core sequence which is a close copy of the central region of the promoter (4, 53) was an early clue that the 60/81-bp elements were involved in transcriptional regulation. Since

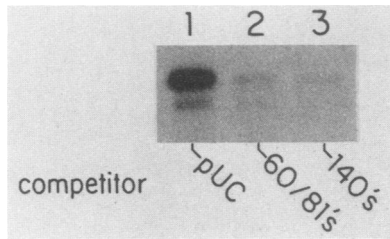


FIG. 6. *trans* competition of *X. laevis* ribosomal gene transcription by mouse 140-bp repeats. *X. borealis* oocytes were coinjected with a plasmid carrying an *X. laevis* ribosomal gene promoter (the A gene described in reference 44; a 5'-245 promoter) and an equal amount of the following plasmids: pUC18 (lane 1); a subcloned block of 10 *X. laevis* 60/81-bp enhancers (lane 2; the E plasmid of reference 44); and a subcloned block of mouse 140-bp repeats (lane 3; the fragment from -1800 to -168 shown in Fig. 1). Six hours later, RNA was extracted and analyzed by S1 nuclease protection.

the mouse 140-bp repeats (28) have no obvious sequence similarity either to the mouse gene promoter (37) or to the frog 60/81-bp repeats, their recognition as transcription elements was delayed.

The present work is the first demonstration that repetitive, *Xenopus*-type enhancers are present in the intergenic spacer of a mammal, and the analogy fits in all respects. Both mouse and frog enhancers can stimulate a promoter *in cis*, and they function *in vivo* in either orientation and in the absence or presence of a competitor template. The mouse enhancers even have the striking ability to enhance in a completely heterologous system, i.e., joined to a *Xenopus* promoter and transcribed in a *Xenopus* cell. In addition, the enhancers of both species compete with promoters *in trans*, bind UBF from either species, and are located between the spacer promoter and the gene promoter.

Probable widespread occurrence of *Xenopus*-type ribosomal gene enhancers. Initially, *X. laevis* and, by analogy, *X. borealis* (50) were the only organisms for which polymerase I enhancers had been described. Thus one could have questioned whether such enhancers were peculiar to amphibian ribosomal genes or whether they were an example of a more general polymerase I regulatory mechanism. However, evidence for *Xenopus*-type enhancers in other species has been accumulating.

The first two lines of evidence came from studies of nucleolar dominance and of sequence organization. In *Xenopus* species, nucleolar dominance between closely related species is evidently due to competition between ribosomal genes bearing unequal numbers of enhancers (50). The fact that nucleolar dominance is widespread among eucaryotes (reviewed in reference 49) argues that enhancers of the *Xenopus* type will also be widespread. In fact, many organisms, including plants (see reference 1 and references therein), have genetically unstable repeated elements just upstream of the ribosomal gene promoter, and in wheat (35) these elements have been implicated in causing nucleolar dominance. In rats (8) and Chinese hamsters (14) these repeats have substantial sequence homology to the mouse enhancers. The analogous region of the human ribosomal gene spacer, while not obviously repetitive, does contain multiple copies of a core region of the rodent element, and these copies are at approximately the same relative positions and have about the same periodicity as the elements in the rodent repeats (14). It is possible that these repeated elements will turn out to be polymerase I enhancers.

In *Drosophila melanogaster*, repetitive elements in the

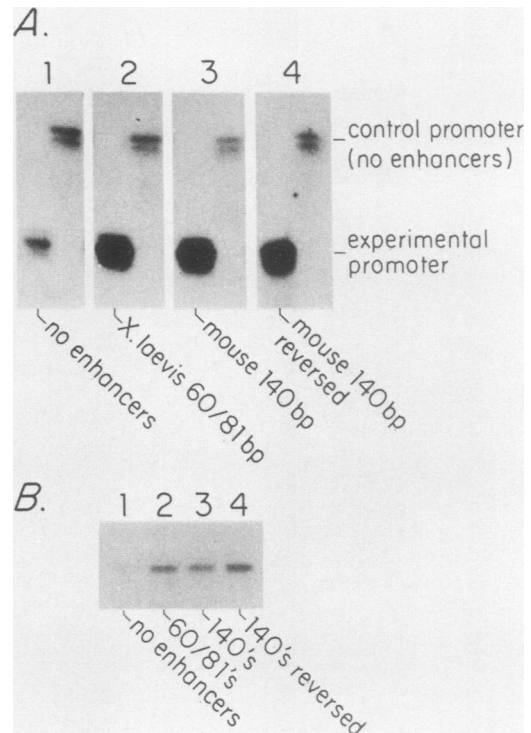


FIG. 7. Mouse 140-bp repeats enhance frog ribosomal gene promoters. (A) *X. laevis* oocytes were injected with equimolar amounts of a control promoter plasmid lacking enhancers (pseudo 52 [30]) plus one of the following plasmids: pseudo 40 (lane 1; another control promoter lacking enhancers); pseudo 40 attached to a block of 10 *X. laevis* enhancers (lane 2); pseudo 40 attached to a block of mouse 140-bp repeats (lane 3); pseudo 40 attached to a block of 140-bp repeats in the reverse orientation (lane 4). In lanes 1, each oocyte received a total of 500 pg of DNA; the other lanes were equimolar with lanes 1. Following overnight incubation, RNA was extracted and analyzed by S1 protection by using a probe specific for the transcript from the experimental promoter in the first track of each set of lanes and a probe specific for the transcript of the enhancerless control promoter in the second track of each set of lanes. (B) *X. borealis* oocytes were singly injected with equimolar amounts of the same series of plasmids used in panel A in the absence of pseudo 52 competitor: pseudo 40 (lane 1); pseudo 40 attached to the *X. laevis* enhancers (lane 2); pseudo 40 attached to the mouse 140-bp repeats (lane 3); pseudo 40 attached to the 140-bp repeats in the reverse orientation (lane 4). In lanes 3 and 4, each oocyte received 1.5 ng of DNA. In all other lanes, the total DNA amount was brought up to this level by the addition of pUC18 vector DNA. After 6 h of incubation, RNA was isolated and analyzed by S1 nuclease protection.

intergenic spacer have been shown to stimulate a ribosomal gene promoter *in cis* in proportion to the number of elements (21), a situation analogous to that with *Xenopus*-type enhancers. However, the *Drosophila* elements, unlike the *Xenopus* and mouse enhancers, stimulate transcription only in the forward orientation and not in the reverse orientation, and they are duplications of the polymerase I promoter. Although it is not clear how these elements function and whether they have an enhancer effect distinct from their function as promoters, they could be *Xenopus*-type enhancers. The conserved arrangement of spacer regulatory elements in mammals, amphibians, and possibly plants and insects implies that this arrangement has important functional significance for ribosomal genes.

In yeast cells, the ribosomal gene spacer contains an element which has a strong stimulatory effect on a ribosomal gene promoter located in *cis*. Although not obviously repetitive, this yeast element strongly resembles the *Xenopus*- and mouse-type enhancers in that it functions in vivo, acts in both orientations and over considerable distances (17), resides within a polymerase I transcription unit, and binds a protein factor which also binds near the gene promoter (39). It would not be surprising if this yeast enhancer and *Xenopus*-type enhancers turn out to operate by the same fundamental mechanism.

Comparison with reports of other mammalian polymerase I enhancers. Initiation at the ribosomal gene promoter can be stimulated by many different sequence elements operating via several distinct mechanisms. Stimulation can result not only from enhancers, but also from terminators (11, 25, 26, 36), spacer promoters (10), and high-mobility-group-like protein binding sites (58). We would like to see the term enhancer reserved for those in vivo stimulatory elements which are analogous to the *Xenopus* and mouse enhancers and which match the criteria listed at the beginning of this discussion.

Two elements in the rat spacer have been previously reported to be enhancers, but neither fits the above-mentioned criteria very well. One of these elements (12, 13, 20) is 37 bp in length and not obviously repetitive, and it resides upstream of both the known spacer promoter (7) and the rat analog of the mouse 140-bp repeats. Since this element has been reported to stimulate in S-100-type extracts in vitro (it has not been tested in vivo), to stimulate polymerase II as well as polymerase I promoters, and to stimulate nonspecific initiation and specific initiation approximately equally, it appears to be distinct from enhancer action as defined above. The other reported rat enhancer is in a 735-bp region of the spacer immediately upstream of the gene promoter (6) and has been observed to stimulate in vitro rDNA transcription by up to 17-fold in S-100 extracts (it also has not been examined in vivo). This is a more complex situation, since this 735-bp region contains three different kinds of elements: (i) a binding site for a high-mobility-group-like protein which clearly is responsible for at least part of the in vitro stimulation (57), (ii) a spacer promoter (7), and (iii) three copies of the genetically unstable repetitive element which is homologous to the mouse 140-bp enhancer repeats. However, it seems unlikely that the 140-bp enhancer repeats contributed to the in vitro stimulation, since the mouse and frog enhancers did not show *cis* stimulation in such in vitro extracts.

Isolation of a UBF homolog from mice. This article demonstrates the existence of UBF in mouse cells (mUBF). UBF homologs have now been purified from humans (UBF1 or hUBF; 2, 34), *X. laevis* (xUBF; 3, 47), rats (48a), and mice (this work). All of the mammalian UBFs have the same electrophoretic size, while frog UBF is about 10 kilodaltons smaller. A factor TFIS isolated from frog (15) contains xUBF since its two largest polypeptides coelectrophorese with the xUBF polypeptides (M. Dunaway and C. Pikaard, unpublished data). Human, frog, and rat UBFs have been shown to stimulate transcription by RNA polymerase I in homologous transcription systems (2, 47; Pikaard et al., submitted). It seems a safe prediction that mUBF will also have a transcriptional effect in mice, and recent experiments indicate that this indeed is the case (K. Ryan, L. Pape, and B. Sollner-Webb, unpublished data).

The most distinctive feature of UBF proteins is that, regardless of their species of origin, they all exhibit virtually identical DNA-binding properties, thus causing characteris-

tic DNase I footprints, with extensive regions of reduced cleavage punctuated by discrete sites of enhanced cleavage (e.g., Fig. 5). The likelihood is that this common DNA binding will turn out to be a distinguishing feature of all vertebrate UBFs. This conservation of binding properties is surprising, since both promoter and enhancer sequences have diverged widely from amphibians to mammals. Evidently, UBF can recognize different sequences which are generally GC rich but do not show a simple conserved sequence. On the basis of five UBF-binding regions, a weak consensus sequence (C/T G G G C/G A/C G) was suggested (47), but neither the mammalian core enhancer sequence (14; underlined in Fig. 4A) nor even the entire 140-bp mouse enhancer precisely matches this sequence. The relatively relaxed sequence specificity of UBF is in accord with the recent finding that the DNA-binding domains of hUBF are related to those of the relatively abundant chromosomal proteins HMG1 and HMG2 (27). Until we better understand what defines a UBF-binding site, it will not be possible to locate potential enhancer or promoter sequences by computer search alone.

The ability of UBFs from various species to generate identical footprints leads to the conclusion that the well-known species specificity of polymerase I transcription (9, 24) is not due to divergence in DNA recognition by UBF (3) and that it thus must be caused at some other level(s). Three different mechanisms for species specificity have experimental support. (i) Other polymerase I transcription factors can exhibit species-specific interactions with promoter sequences, as has been shown for mouse factor D (38, 55). (ii) Protein-protein interactions with other factors can diverge, as observed for the human SL1-UBF interaction (3). (iii) Critical changes in the spacing between promoter domains can occur during speciation, as shown for *X. laevis* species and mice, in which the relative orientations of the upstream and core domains differ by half a helix turn (45). It is likely that the above-described mechanisms for species specificity are not mutually exclusive or even exhaustive.

Implications for mechanism of enhancer function. Although mouse and frog promoters exhibit species specificity when assayed in vivo, mouse enhancers function as well in a frog oocyte when attached to a frog promoter as do the homologous frog enhancers (Fig. 6). This suggests that the promoter has a more complicated task to perform than does the enhancer. In addition to binding UBF, it must coordinate the correct interaction with at least one other factor and it must position the polymerase for accurate initiation. In contrast, the primary role of an RNA polymerase I enhancer might be only to bind UBF. This suggests that enhancer function, along with UBF binding, may be widely conserved across species even though promoter function is considerably more species selective.

The ideas described above also lead to the hypothesis that polymerase I enhancers initially arose as spontaneous tandem duplications of promoter sequences (UBF-binding sites). In some organisms, like *D. melanogaster*, the enhancers created by such duplication retained their ability to function as promoters. In other cases, such as in frogs, only a part of the promoter has been duplicated. In yet other cases, as in mice, the derivation of the enhancer repeats is not obvious, but the repeats are clearly UBF-binding sites. Notably, in those instances in which only a part of the promoter or other sequences has been duplicated, one or more intact promoters (spacer promoters) are also present upstream of the enhancers. This indicates that it is important

to have the capability to transcribe through the enhancer repeats.

It may be that the polymerase I enhancers represent a prototypical enhancer which has as its only task the attraction of transcription factors that also bind the promoter, thereby increasing their concentration in the vicinity of the gene promoter. Polymerase II enhancers may have evolved from a similar prototype, since many of them share binding domains for essential transcription factors with their cognate promoters (18, 33, 46). However, polymerase II enhancers additionally respond to a wide variety of cell cycle, tissue-specific, or other signals that the basic polymerase II promoters do not respond to. This may account for the number of other factor-binding sites that they often contain and that may overlie the elementary enhancer.

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ADDENDUM IN PROOF

While this article was in press, mouse UBF was also reported by S. Bell, H.-M. Jantzen, and R. Tjian (*Genes Dev.* 4:943-954, 1990).

LITERATURE CITED

- Barker, R. F., N. P. Harberd, M. G. Jarvis, and R. B. Flavell. 1988. Structure and evolution of the intergenic region in a ribosomal DNA repeat unit of wheat. *J. Mol. Biol.* 201:1-17.
- Bell, S. P., R. M. Learned, H.-M. Jantzen, and R. Tjian. 1988. Functional cooperativity between transcription factors UBF1 and SL1 mediates human ribosomal RNA synthesis. *Science* 241:1192-1197.
- Bell, S. P., C. S. Pikaard, R. H. Reeder, and R. Tjian. 1989. Molecular mechanisms governing species specific transcription of ribosomal RNA. *Cell* 59:489-497.
- Boseley, P., T. Moss, M. Machler, R. Portmann, and M. Birnstiel. 1979. Sequence organization of the spacer DNA in a ribosomal gene unit of *X. laevis*. *Cell* 17:19-31.
- Busby, S. J., and R. H. Reeder. 1983. Spacer sequences regulate transcription of ribosomal gene plasmids injected into *Xenopus* embryos. *Cell* 34:989-996.
- Cassidy, B. G., H. F. Yang-Yen, and L. I. Rothblum. 1986. Transcriptional role for the nontranscribed spacer of rat ribosomal DNA. *Mol. Cell. Biol.* 6:2766-2773.
- Cassidy, B. G., H.-F. Yang-Yen, and L. I. Rothblum. 1987. Additional RNA polymerase I initiation site within the nontranscribed spacer region of the rat rRNA gene. *Mol. Cell. Biol.* 7:2388-2396.
- Chikaraishi, D. M., L. Buchanan, K. J. Danna, and C. A. Harrington. 1983. Genomic organization of rat rDNA. *Nucleic Acids Res.* 11:6437-6452.
- Culotta, V., J. Wilkinson, and B. Sollner-Webb. 1987. Mouse and frog violate the paradigm of species-specific ribosomal RNA transcription. *Proc. Natl. Acad. Sci. USA* 84:7498-7502.
- DeWinter, R. F. J., and T. Moss. 1986. Spacer promoters are essential for efficient enhancement of *X. laevis* ribosomal transcription. *Cell* 44:313-318.
- DeWinter, R. F. J., and T. Moss. 1987. A complex array of sequences enhances ribosomal transcription in *Xenopus laevis*. *J. Mol. Biol.* 196:813-827.
- Dixit, A., L. C. Garg, W. Chao, and S. T. Jacob. 1987. An enhancer element in the far upstream spacer region of rat ribosomal RNA gene. *J. Biol. Chem.* 262:11616-11622.
- Dixit, A., L. C. Garg, and S. T. Jacob. 1989. A cis-acting sequence within the rat ribosomal DNA enhancer region can modulate RNA polymerase II-directed transcription of the metallothionein gene in vitro. *DNA* 8:311-320.
- Dumenco, V. M., and P. J. Wejksnora. 1986. Characterization of the region around the start point of transcription of ribosomal RNA in the Chinese hamster. *Gene* 46:227-235.
- Dunaway, M. 1989. A transcription factor, TFIS, interacts with both the promoter and enhancer of the *Xenopus* rRNA genes. *Genes Dev.* 3:1768-1778.
- Dunaway, M., and P. Droge. 1989. Transactivation of the *Xenopus* rRNA gene promoter by its enhancer. *Nature (London)* 341:657-659.
- Elion, E. A., and J. R. Warner. 1986. An RNA polymerase I enhancer is *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 6:2089-2097.
- Evans, T., M. Reitman, and G. Felsenfeld. 1988. An erythrocyte-specific DNA-binding factor recognizes a regulatory sequence common to all chicken globin genes. *Proc. Natl. Acad. Sci. USA* 85:5976-5980.
- Galas, D., and A. Schmitz. 1978. DNase footprinting: a simple method for the detection of protein-DNA binding specificity. *Nucleic Acids Res.* 5:3157-3170.
- Garg, L. C., A. Dixit, and S. T. Jacob. 1989. A 37-base pair element in the far upstream spacer region can enhance transcription of rat rDNA in vitro and can bind to the core promoter-binding factor(s). *J. Biol. Chem.* 264:220-224.
- Grimaldi, G., and P. P. Di Nocera. 1988. Multiple repeated units in *Drosophila melanogaster* ribosomal DNA spacer stimulate rRNA precursor transcription. *Proc. Natl. Acad. Sci. USA* 85:5502-5506.
- Grummt, I., A. Kuhn, I. Bartsch, and H. Rosenbauer. 1986. A transcription terminator located upstream of the mouse rDNA initiation site affects rRNA synthesis. *Cell* 47:901-911.
- Grummt, I., U. Maier, A. Ohrlein, A. Hassouna, and J.-P. Bachellerie. 1985. Transcription of mouse rDNA terminates downstream of the 3' end of 28S RNA and involves interaction of factors with repeated sequences in the 3' spacer. *Cell* 43:801-810.
- Grummt, I., E. Roth, and M. Paule. 1982. Ribosomal RNA transcription in vitro is species-specific. *Nature (London)* 296:173-174.
- Henderson, S., and B. Sollner-Webb. 1986. A transcriptional terminator is a novel element of the promoter of the mouse ribosomal RNA gene. *Cell* 47:891-900.
- Henderson, S. K., K. Ryan, and B. Sollner-Webb. 1989. The promoter-proximal rDNA terminator augments initiation by preventing disruption of the stable transcription complex caused by polymerase read-in. *Genes Dev.* 3:212-223.
- Jantzen, H. M., A. Admon, S. P. Bell, and R. Tjian. 1990. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature (London)* 344:830-836.
- Kuehn, M., and N. Arnheim. 1983. Nucleotide sequence of the genetically labile repeated elements 5' to the origin of mouse rRNA transcription. *Nucleic Acids Res.* 11:211-224.
- Kuhn, A., and I. Grummt. 1987. A novel promoter in the mouse rDNA spacer is active in vivo and in vitro. *EMBO J.* 6:3487-3492.
- 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.
- 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.
- Labhart, P., and R. H. Reeder. 1989. A point mutation uncouples RNA 3' end formation and termination during ribosomal gene transcription in *X. laevis*. *Genes Dev.* 4:269-276.
- Landschultz, W. H., P. F. Johnson, E. Y. Adashi, B. J. Graves, and S. L. McKnight. 1988. Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* 2:786-800.
- Learned, R. M., T. K. Learned, M. M. Haltiner, and R. T. Tjian. 1986. Human rRNA transcription is modulated by the coordi-

- nate binding of two factors to an upstream control element. *Cell* **45**:847–857.
35. **Martini, G., M. O'Dell, and R. B. Flavell.** 1982. Partial inactivation of wheat nucleolus organizers by the nucleolus organizer chromosomes from *Aegilops umbellulata*. *Chromosoma (Berlin)* **84**:687–700.
 36. **McStay, B., and R. H. Reeder.** 1986. A termination site for *Xenopus* RNA polymerase I also acts as an element of an adjacent promoter. *Cell* **47**:913–920.
 37. **Miller, K., J. Tower, and B. Sollner-Webb.** 1985. A complex control region of the mouse rRNA gene directs accurate initiation by RNA polymerase I. *Mol. Cell. Biol.* **5**:554–562.
 38. **Mishima, Y., I. Financsek, R. Kominami, and M. Muramatsu.** 1982. Fractionation and reconstitution of factors required for accurate transcription of mammalian ribosomal RNA genes: identification of a species-dependent initiation factor. *Nucleic Acids Res.* **10**:6659–6670.
 39. **Morrow, B. E., S. P. Johnson, and J. R. Warner.** 1989. Proteins that bind to the yeast rDNA enhancer. *J. Biol. Chem.* **264**:9061–9068.
 40. **Moss, T.** 1983. A transcriptional function for the repetitive ribosomal spacer in *Xenopus laevis*. *Nature (London)* **302**:223–228.
 41. **Moss, T., and M. Birnstiel.** 1979. The putative promoter of a *Xenopus laevis* ribosomal gene is reduplicated. *Nucleic Acids Res.* **6**:3733–3743.
 42. **Moss, T., K. Mitchelson, and R. F. J. DeWinter.** 1985. The promotion of ribosomal transcription in eukaryotes, p. 207–250. *In* N. McLean (ed.), *Oxford surveys on eukaryotic genes*, vol. 2. Oxford University Press, Oxford.
 43. **Murtif, V. L., and P. M. M. Rae.** 1985. *In vivo* transcription of rDNA spacers in *Drosophila*. *Nucleic Acids Res.* **13**:3221–3239.
 44. **Pape, L. K., J. J. Windle, and B. Sollner-Webb.** 1989. The *Xenopus* rDNA 60/81-bp repeats direct true *cis*-enhancement and function at the establishment of the preinitiation complex: analysis *in vitro* and in an enhancer-responsive *in vitro* system. *Mol. Cell. Biol.* **9**:5093–5104.
 45. **Pape, L. K., J. J. Windle, and B. Sollner-Webb.** 1990. Half helix turn spacing changes convert a frog into a mouse rDNA promoter: a distant upstream domain determines the helix face of the initiation site. *Genes Dev.* **4**:52–62.
 46. **Peterson, C. L., S. Eaton, and K. Calame.** 1988. Purified gamma EBP-E binds to immunoglobulin enhancers and promoters. *Mol. Cell. Biol.* **8**:4972–4980.
 47. **Pikaard, C. S., B. McStay, M. C. Schultz, S. P. Bell, and R. H. Reeder.** 1989. The *Xenopus* ribosomal gene enhancers bind an essential polymerase I transcription factor, xUBF. *Genes Dev.* **3**:1779–1788.
 48. **Pikaard, C. S., and R. H. Reeder.** 1988. Sequence elements essential for function of the *Xenopus laevis* ribosomal DNA enhancers. *Mol. Cell. Biol.* **8**:4282–4288.
 - 48a. **Pikaard, C. S., S. D. Smith, R. H. Reeder, and L. Rothblum.** 1990. rUBF, an RNA polymerase I transcription factor from rats, produces DNase I footprints identical to those produced by xUBF, its homolog from frogs. *Mol. Cell. Biol.* **10**:3810–3812.
 49. **Reeder, R. H.** 1985. Mechanisms of nucleolar dominance in animals and plants. *J. Cell Biol.* **101**:2013–2016.
 - 49a. **Reeder, R. H.** 1989. Regulatory elements of the generic ribosomal genes. *Curr. Opin. Cell Biol.* **1**:466–474.
 50. **Reeder, R. H., and J. G. Roan.** 1984. The mechanism of nucleolar dominance in *Xenopus* hybrids. *Cell* **38**:39–44.
 51. **Reeder, R. H., J. G. Roan, and M. Dunaway.** 1983. Spacer regulation of *Xenopus* ribosomal gene transcription: competition in oocytes. *Cell* **35**:449–456.
 52. **Sollner-Webb, B., and S. McKnight.** 1982. Accurate transcription of cloned *Xenopus* ribosomal RNA genes. *Nucleic Acids Res.* **10**:3391–3405.
 53. **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.
 54. **Sollner-Webb, B., and J. Tower.** 1986. Transcription of cloned eukaryotic ribosomal RNA genes. *Annu. Rev. Biochem.* **55**:801–830.
 55. **Tower, J., V. Culotta, and B. Sollner-Webb.** 1986. The factors and nucleotide sequences that direct ribosomal DNA transcription and their relationship to the stable transcription complex. *Mol. Cell. Biol.* **6**:3451–3462.
 56. **Tower, J., S. H. Henderson, K. M. Dougherty, P. J. Wejksnora, and B. Sollner-Webb.** 1989. An RNA polymerase I promoter located in the CHO and mouse ribosomal DNA spacer: functional analysis and factor and sequence requirements. *Mol. Cell. Biol.* **9**:1513–1525.
 57. **Wilkinson, J. J., and B. Sollner-Webb.** 1982. Transcription of *Xenopus* ribosomal RNA genes by RNA polymerase I *in vitro*. *J. Biol. Chem.* **257**:14375–14383.
 58. **Yang-Yen, H.-F., and L. I. Rothblum.** 1988. Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis *in vitro*. *Mol. Cell. Biol.* **8**:3406–3414.