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rUBF, an RNA Polymerase I Transcription Factor from Rats, Produces DNase I Footprints Identical to Those Produced by xUBF, Its Homolog from Frogs

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Rat cells contain a DNA-binding polymerase I transcription factor, rUBF, with properties similar to UBF homologs that have been purified from both human (hUBF) and frog (xUBF) cells. In this note we report the affinity purification of rUBF to apparent homogeneity and show that UBFs from both rat and frog have identical footprinting characteristics on templates from either species. Furthermore, xUBF was able to stimulate transcription from rat RNA polymerase I promoters in a partially fractionated rat extract that was UBF dependent. These results strengthen the conclusion that all vertebrate cells contain a UBF homolog whose DNA-binding specificity and function have been strongly conserved.

Promoters for RNA polymerase I are generally found to be highly species specific, in contrast to the extensive cross-species functioning of many promoters for RNA polymerase II and III. The species specificity of polymerase I promoters also correlates with the lack of any easily recognizable conservation between the promoter sequences of most species.

Recently, a transcription factor that binds to the human ribosomal gene promoter hUBF has been purified from human cells (1). Subsequently, the homologous polymerase I transcription factor from *Xenopus laevis*, xUBF, was also purified (5). Comparison of hUBF and xUBF has led to the surprising observation that they produce identical DNase I footprints on templates from either species (2, 5). Thus, the UBF DNA-binding specificity has been strongly conserved from frogs to humans, and the sequence motifs in the promoters must be more conserved than is readily apparent. Furthermore, the molecular cause of species specificity must reside in some other part of the transcription machinery, not in the UBF promoter recognition (2). Fractionation of mouse extracts reveals that they also contain a UBF homolog (mUBF [C. S. Pikaard, L. K. Pape, S. L. Henderson, K. Ryan, M. H. Paalman, M. A. Lopata, R. H. Reeder, and B. Sollner-Webb, submitted for publication]), which again has footprinting characteristics identical to those of xUBF and hUBF.

In this article we show that a UBF homolog can also be purified from rat cells. Rat UBF (rUBF) also produces DNase I footprints identical to those produced by xUBF, strengthening the conclusion that a UBF homolog with identical DNA-binding specificity will be found in all vertebrate cells. We also examined the ability of rUBF and xUBF to substitute for each other during in vitro transcription. We have been unable to detect any functioning of rUBF in *X. laevis* extracts. However, xUBF will substitute for rUBF and allow a rat promoter to be transcribed in a UBF-dependent rat extract.

The fractionation of extracts from rat cells and the identification of a fraction with the properties of UBF have been described elsewhere (5a). A summary of the fractionation scheme is shown in Fig. 1A. Final purification of rUBF was achieved by passing the CM-300 fraction over a DNA affinity column containing sequences of the *X. laevis* ribosomal gene enhancers. Elution of the affinity column with 0.6 M KCl yielded a fraction containing two closely spaced polypep-

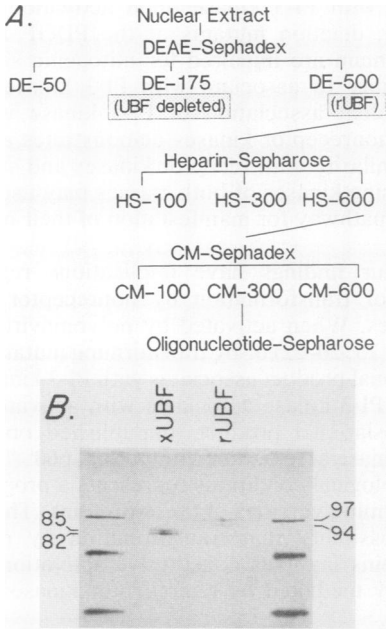

FIG. 1. Purification of rat UBF. (A) Purification scheme. Nuclear extract, prepared as described by Haglund and Rothblum (4), was fractionated by the scheme shown. The transcriptional properties of each of the various fractions is described by Smith et al. (5a). Final purification was achieved by dialyzing the CM-300 fraction against 100 mM KCl, passing it over a DNA affinity column containing *X. laevis* ribosomal gene enhancer sequences (5), and eluting the bound material with 600 mM KCl. (B) SDS gel electrophoresis. rUBF, the 600 mM KCl eluate from the DNA affinity step, was electrophoresed on an SDS-acrylamide gel, and the gel was silver stained. Only two bands 94 and 97 kDa are visible. Lane xUBF, Affinity-purified UBF from *X. laevis*, run for comparison with rUBF. Outer lanes contain size markers.

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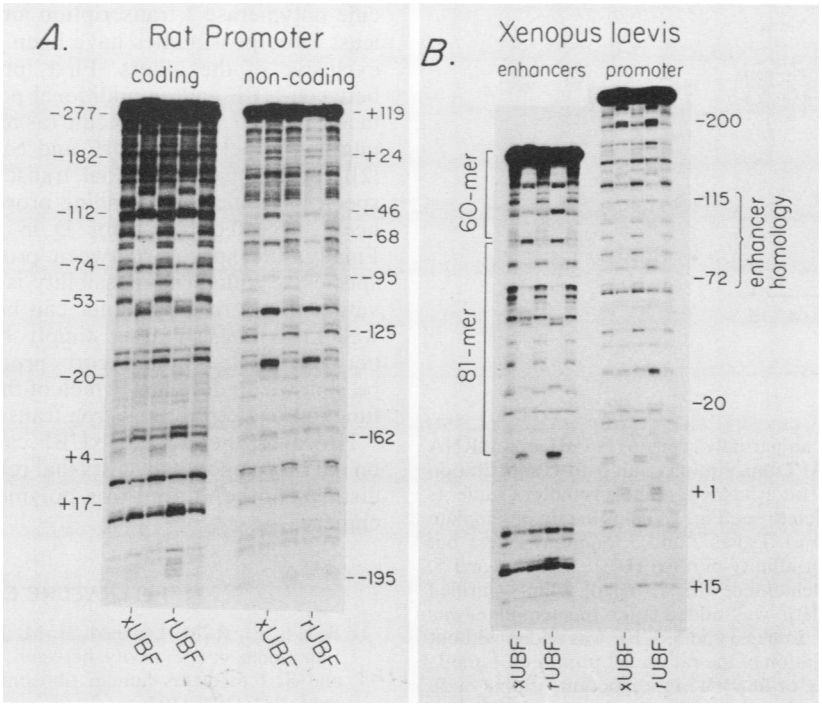

FIG. 2. Rat and *Xenopus* UBFs produce nearly identical DNase I footprints. (A) DNase I footprints on the coding and noncoding strands of the rat ribosomal gene promoter. The coordinates give nucleotide position relative to the site of transcription initiation. (B) DNase I footprints on the *X. laevis* enhancers and the *X. laevis* ribosomal gene promoter. In all cases footprinting was done with the affinity-purified UBF preparations shown in Fig. 1B. Middle and outer lanes contain naked DNA.

tides of 94 and 97 kilodaltons (kDa) and nothing else that was visible on a silver-stained sodium dodecyl sulfate (SDS)-acrylamide gel (Fig. 1B). The appearance of UBF as two closely spaced bands is typical of proteins that have been purified from human (1), frog (5), and mouse (Pikaard et al., submitted), and the estimated molecular mass is identical to that of hUBF (xUBF is about 10 kDa smaller than the mammalian factors).

Affinity-purified rUBF (same preparation as shown in Fig. 1B) was used in DNase I footprinting along with affinity-purified xUBF on a variety of templates from both rat and frog. Two examples are shown in Fig. 2. The ability of rUBF and xUBF to footprint on the rat promoter is shown in Fig. 2A. On the coding strand, rUBF and xUBF produced nearly identical footprints. There was a region of strong protection from about −50 to −130, with hypersensitive bands at approximately 20-base-pair (bp) intervals. Small but reproducible alterations in the digestion pattern were also seen around the initiation site at +1. It is striking that an almost identical pattern of protection and hypersensitivity was also observed on the noncoding strand.

UBFs from both species were used to footprint on the *X. laevis* ribosomal gene enhancers as well as on the *X. laevis* gene promoter (Fig. 2B). Again, both species of UBF produced very similar footprints. On the enhancers, protection extended over each element, whether it was an 81-mer or a 60-mer, with strong hypersensitive sites at the boundaries of elements. Footprinting on the promoter was more complex, but the most extensive protection appeared over the enhancer homology region. As on the rat promoter, minor perturbations were also seen in the region around +1. A more extensive discussion of the UBF footprints on *X. laevis* templates has been published elsewhere (5).

From the results shown in Fig. 2 it is apparent that rUBF has DNase I footprinting specificity that is nearly identical to that of xUBF. Thus, this particular DNA-binding specificity has been conserved among human, mouse, rat, and frog and is probably conserved among all vertebrates. Since rUBF binds to the *X. laevis* enhancer elements in the same manner as xUBF, this suggests that rat rDNA may also contain repetitive elements with enhancer function. This suggestion is strengthened by the recent observation that mouse rDNA does contain repetitive elements with enhancer activity and which bind UBF (Pikaard et al., submitted).

Although the DNA-binding specificity of UBF has been strongly conserved among the vertebrates, the ability of UBF from one species to replace the UBF from another in a transcription reaction appears to be limited. For example, neither xUBF nor hUBF was able to substitute for the other in transcription systems from human and frog, respectively (2). Similarly, in a frog transcription system depleted for xUBF, we have been unable to obtain any stimulation of initiation from a frog promoter by addition of rUBF (data not shown). In contrast, purified xUBF caused significant stimulation of initiation from a rat promoter in a UBF-depleted rat extract. This result is shown in Fig. 3 for a mutant of the gene promoter (an A to G substitution at position −16) as well for the wild-type spacer promoter from rat rDNA.

The transcription extract used for the experiments in Fig. 3 was the DE-175 fraction (see Fig. 1A), which contains all the components required for specific polymerase I transcription but is depleted of rUBF. In this depleted extract, transcription of both the mutated gene promoter as well as the spacer promoter was completely dependent on the addition of rUBF (compare Fig. 3A, lane 1, with lanes 2 and 3; in Fig. 3B, compare lane 1 with lane 2). rUBF is probably not completely absent from the DE-175 fraction, since this fraction supported a low level of transcription from the

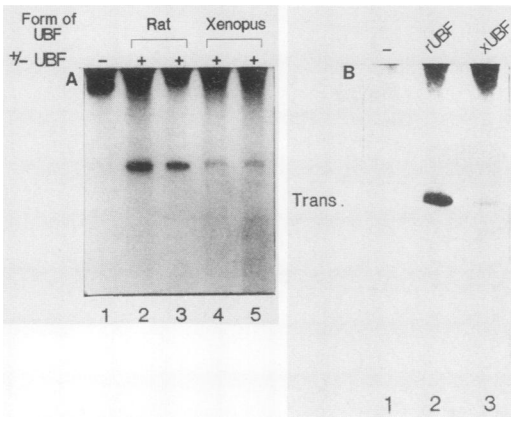

FIG. 3. *Xenopus* UBF can partially replace rat UBF on rat RNA polymerase I promoters. (A) Transcription of an A-to-G substitution mutant (−16 A/G) of the rat ribosomal gene promoter. Lane 1, DE-175 fraction (UBF depleted; see Fig. 1A) plus 0.4 μg of template in a reaction volume of 50 μl. Lanes 2 and 3, Same as lane 1 but supplemented with 10 ng of affinity-purified rUBF. Lanes 4 and 5, Same as lane 1 but supplemented with 10 ng of affinity-purified xUBF. Lanes 2 and 4, UBF was added after the template was incubated with the DE-175. Lanes 3 and 5, UBF was added without preincubation. (B) Transcription of the rat spacer promoter. Lane 1, DE-175 fraction plus 0.2 μg of template in a reaction volume of 50 μl. Lane 2, Same as lane 1 but supplemented with rUBF (the CM-300 fraction [see Fig. 1A]; an amount was used that had footprinting activity similar to the 10 ng of xUBF added in lane 3). Lane 3, Same as lane 1 but supplemented with 10 ng of affinity-purified xUBF. Trans., Transcript.

wild-type gene promoter without supplementation (data not shown; described more fully by Smith et al. [5a]). However, for our present purpose, the important fact is that transcription of either the mutated gene promoter or the spacer promoter was completely dependent upon supplementation with rUBF.

Transcription of the mutated gene promoter was also stimulated by the addition of affinity-purified xUBF (Fig. 3A, lanes 4 and 5). Likewise, Fig. 3B, lane 3, shows that the spacer promoter was also stimulated by xUBF. For both types of promoter we were unable to increase transcription by addition of more xUBF. Therefore, xUBF apparently cannot replace rUBF completely on a rat promoter, but there was significant cross-reaction. The fact that xUBF can partially substitute for rUBF is further evidence that these two transcription factors are homologs. A previous instance of cross-reaction between rodent and amphibian polymerase I transcription apparatus has been reported, in which a frog promoter was recognized by a mouse extract (7).

If the UBF-DNA interaction has been highly conserved, what is the molecular mechanism that causes species-specific polymerase I transcription among the vertebrates? At least three possibilities have been suggested, none of them exclusive of the others. First, protein-protein interaction between UBF and an additional polymerase I transcription factor may be species specific (as has been suggested for the interaction between hUBF and SL1 in the human system [2]). Second, an additional transcription factor may have species-specific DNA-binding properties of its own (as has been suggested for factor D in the mouse system [6]). Finally, the spacing between promoter domains may be species specific. This possibility is supported by the observation that a frog promoter can be made to function very well in a mouse extract simply by changing the spacing between upstream and core promoter domains (4a). At present we do not know which of these or other mechanisms function to keep rat and frog transcription species specific.

However, the fact that xUBF can partially replace rUBF on the rat promoter suggests that relatively minor differences distinguish the rat and frog polymerase I transcription machinery.

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