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Dual role of the nucleolar transcription factor UBF: Trans-activator and antirepressor

(ribosomal genes/transcription factors/RNA polymerase I/repression/transactivation)

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ABSTRACT **In a reconstituted system consisting of partially purified RNA polymerase I (pol I) and the initiation factors TIF-IA, TIF-IB, and TIF-IC, the nucleolar factor UBF (upstream binding factor) stimulates transcription from the rRNA-encoding DNA (rDNA) promoter at least 50-fold. This activation is not observed at high template concentrations or in the presence of highly purified pol I. Template commitment experiments suggest that UBF activates transcription by relieving inhibition exerted by a negative-acting factor(s) in the polymerase fraction that competes for TIF-IB binding to the rDNA promoter and prevents the formation of preinitiation complexes. Using purified histone H1 bound to DNA as a model for the repressed state of the rDNA promoter, we show that UBF counteracts H1-mediated repression of pol I transcription. The implications of these findings are discussed with respect to the protein–protein and protein–DNA interactions at the rDNA promoter and the possible involvement of UBF in control of ribosomal gene transcription.**

Transcription initiation by DNA-dependent RNA polymerase I (pol I) from the ribosomal gene promoter is a multistage process requiring the action of at least four initiation factors—called in the mouse system TIF-IA, TIF-IB, TIF-IC, and mUBF—which assemble at the ribosomal gene promoter together with pol I in an ordered fashion to form active preinitiation complexes (1). Transcription specificity is brought about by TIF-IB (2, 3), which forms a strong cooperative complex at the rDNA promoter together with another DNA-binding protein, designated UBF for upstream binding factor (4). Although cDNAs encoding UBF have been cloned and domains involved in dimerization and DNA binding have been identified (5, 6), the mechanism of how UBF activates transcription has yet to be elucidated.

In this communication we show that, in addition to its transactivating function, UBF serves an additional function before or during assembly of initiation complexes. Using partially purified factor preparations, we have functionally identified a yet-unknown protein that competes with TIF-IB for binding to the rDNA promoter and, thus, represses transcription. Our data show that UBF counteracts this inhibitory factor. According to these results, UBF stimulates transcription by two mechanisms. It increases the binding of TIF-IB to its target sequence and counteracts the repressor. This combination of both positively and negatively acting factors may provide a versatile mechanism that enables the cell to control the number of preinitiation complexes at the rRNA-encoding DNA (rDNA) promoter.

MATERIALS AND METHODS

Purification of Transcription Factors and pol I. The fractionation scheme of the individual proteins required to reconstitute transcription is diagrammed in Fig. 1*A*. Most experiments described here were done with a relatively crude pol I fraction that was obtained by chromatography of extracts on DEAE-Sepharose and heparin Ultrogel (H-400 fraction). Despite the fact that this crude pol I fraction contained detectable amounts of UBF, this fraction usually showed more stimulation by UBF than more highly purified preparations. pol I was supplemented with TIF-IA/TIF-IC that was purified on Q-Sepharose. TIF-IB was obtained by chromatography of the H-600 fraction on a CM-Sepharose and Mono Q column. UBF was purified from either H-400 or H-1000 fractions, as indicated in the diagram. After chromatography on a Mono Q column, UBF was purified on Biorex 70 or on a sequence-specific DNA affinity column, as described (7).

***In Vitro* Transcription Assays.** The template pMrWT containing mouse rDNA from position −170 to +155 was linearized with *Nde* I or *Nar* I to generate 371-nucleotide (nt) or 319-nt transcripts, respectively. Transcription was done in a 25-μl assay containing 6 μl of partially purified pol I, 2 μl of TIF-IB, 2 or 4 μl of TIF-IA/TIF-IC, and various amounts of UBF. The transcripts were analyzed by gel electrophoresis, and the radioactivity in each band was counted by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Reconstitution of an UBF-Responsive Transcription System. Mouse UBF was purified to apparent homogeneity from Ehrlich ascites cells (Fig. 1*A*). The final preparation contained a doublet of 97- and 94-kDa polypeptides (Fig. 1*B*), which corresponds to the size of mammalian UBF polypeptides reported (7). To elucidate the functional role of UBF in rDNA transcription, UBF was added to a reconstituted system containing the rDNA template, pol I, TIF-IA, TIF-IB, and TIF-IC. Most experiments were done with partially purified pol I (H-400 fraction), which contains significant amounts of the pol I-associated factors TIF-IA and TIF-IC. To ensure that the reactions contained saturating amounts of these two factors, the assays were usually complemented with a Q-Sepharose fraction containing TIF-IA and TIF-IC activity. Addition of TIF-IB to this crude pol I fraction was not sufficient to promote specific transcription (Fig. 1*C*, lane 1). However, with UBF a strong concentration-dependent stimulation of transcription was seen. The degree of stimulation linearly increased with the amount of UBF and finally reached a plateau. Under optimal conditions UBF activated transcription 50- to 100-fold.

UBF-Directed Transcription Activation Depends on the Amount of Template DNA and TIF-IB. To study the effect of template concentration on UBF-directed transcription activation, increased amounts of pMrWT/Nde I were incubated

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Abbreviations: pol I, DNA-dependent RNA polymerase I; TIF, transcription initiation factor; UBF, upstream binding factor; rDNA, rRNA-encoding DNA; nt, nucleotide(s).

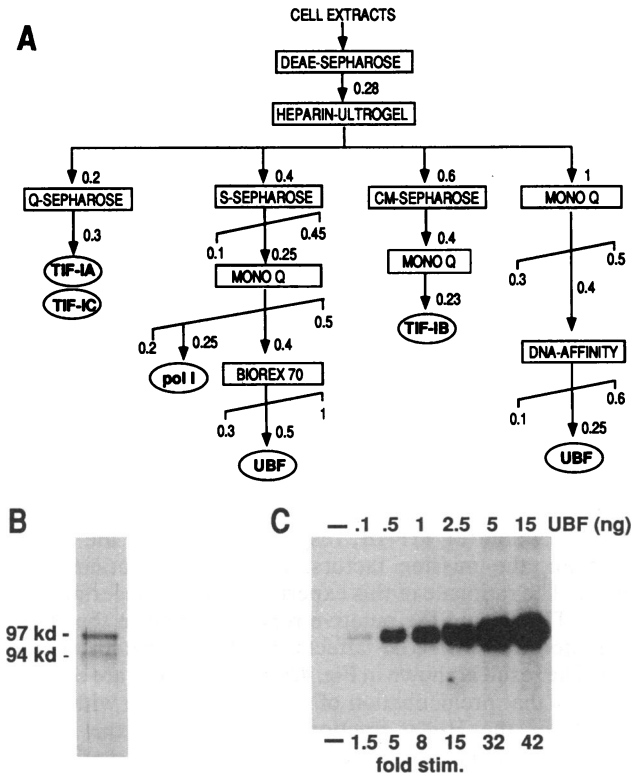


FIG. 1. UBF-dependent rDNA transcription activation. (*A*) Fractionation scheme for purification of UBF and other factors used in the reconstituted transcription system. Numbers indicate molar concentrations of KCl at which individual factor activities eluted from the different columns. (*B*) Silver-stained SDS/polyacrylamide gel of purified UBF. kd, kDa. (*C*) UBF-mediated transcription activation. Reaction mixture contained 2 ng of template DNA (pMrWT/Nde I), 6 μl of pol I (H-400 fraction), 2 μl of TIF-IB, and 2 μl of TIF-IA/TIF-IC. Transcription reactions were done without UBF or with increased amounts of UBF, as indicated above lanes.

together with pol I, TIF-IA, TIF-IC, and TIF-IB with or without UBF (Fig. 2*A*). At low amounts of template transcription absolutely depended on UBF (lanes 1–3). Basal transcription gradually increased with higher template concentrations. Parallel to this template-dependent increase in basal transcription, UBF-induced transcription decreased. This result was unexpected because the pol I fraction used contained detectable amount of UBF. At the highest DNA concentrations tested, transcription rates with and without UBF were identical (lanes 10–12). Moreover, the UBF requirement was overcome by adding increased amounts of TIF-IB (Fig. 2*B*). The finding that UBF did not activate transcription after raising the amount of template DNA or TIF-IB, respectively, suggests that the partially purified reconstituted system contains a yet-unidentified protein that competes for binding of TIF-IB to the promoter. In the absence of UBF the putative inhibitor binds to the promoter and represses transcription. This repression may be relieved either by increasing the amount of template DNA that allows the inhibitor and TIF-IB to distribute on different DNA molecules or by adding UBF. If this hypothesis is correct, then UBF should not stimulate transcription when the template is preincubated with TIF-IB and, therefore, the target site of the inhibitory factor is blocked. Fig. 2*C* shows that this is, indeed, the case. When TIF-IB was added together with the other factors, UBF was required for transcription (lanes 1 and 2). However, when TIF-IB was preincubated with the template before the other factors were added, basal transcription increased, and UBF-directed stimulation declined (lanes 3 and 4).

An Activity in Crude pol I Fractions Is Required for UBF-Dependent Transcription Stimulation. UBF-mediated transcription stimulation not only depends on concentration of the template or amount of TIF-IB but also depends on the individual pol I preparation used. In the experiment of Fig. 3, two different pol I preparations were compared. The pol I in Fig. 3*A* has been partially purified by chromatography on DEAE-Sepharose and heparin-Ultrogel. The pol I in Fig. 3*B* was further purified on S-Sepharose and Mono Q columns. Surprisingly, UBF activated transcription to a much higher extent in the less-purified pol I preparation (Fig. 3*A*) than in the purer one (Fig. 3*B*). The more purified pol I fractions exhibited a relatively high level of basal transcriptional activity, which was only moderately augmented by UBF (compare lanes 1 and 2 in Fig. 3 *A* and *B*). Again, at higher template concentrations UBF did not exert a stimulatory effect. This result indicates that the cruder pol I fraction contains an inhibitory activity that lowers or eliminates basal transcription and that UBF may counteract this repressing activity.

TIF-IB Binding Is Inhibited by a Negative-Acting Factor. To investigate whether the activating function of UBF resides in a step before or after initiation-complex formation and to prove the hypothesis that UBF antagonizes a negative factor in the crude pol I fraction, template commitment experiments were done. The template was cleaved with either *Nde* I or *Nar* I, which yields 371- and 319-nt run-off transcripts, respectively. In the experiment of Fig. 4*A*, the first template (pMrWT/Nde I) was preincubated with different factor combinations before the second template (pMrWT/Nar I), the

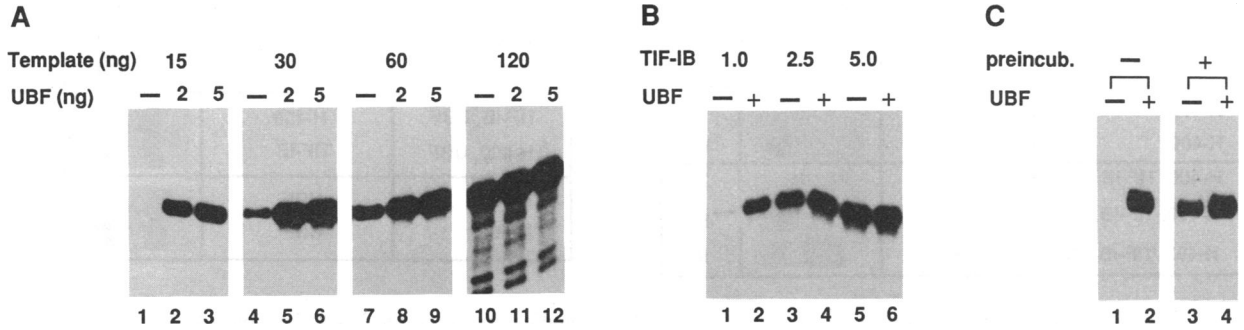


FIG. 2. Effect of DNA and TIF-IB concentrations on UBF-mediated transcription activation. (*A*) Effect of template concentration. Assays contained increased amounts of template DNA, 6 μl of pol I (H-400 fraction), 2 μl of TIF-IB, and 4 μl of TIF-IA/TIF-IC. Reactions were done without UBF or with 2 or 5 ng of UBF, as indicated. (*B*) Effect of TIF-IB amount. Transcription reactions contained 40 ng of pMrWT/Nde I, 6 μl of pol I (H-400), 4 μl of TIF-IA/TIF-IC, and 1.0, 2.5, or 5.0 μl of TIF-IB, respectively. Where indicated, 2 ng of UBF was included in the assays. (*C*) Transcriptions were done as described in *A* with or without 5 ng of UBF. TIF-IB was either added together with the other factors (lanes 1 and 2) or was incubated with the template for 20 min at 30°C before adding the remaining fractions (lanes 3 and 4).

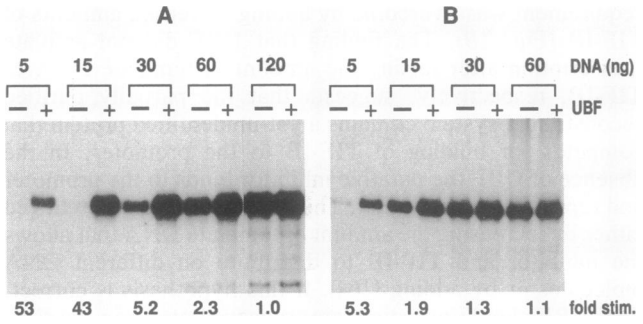
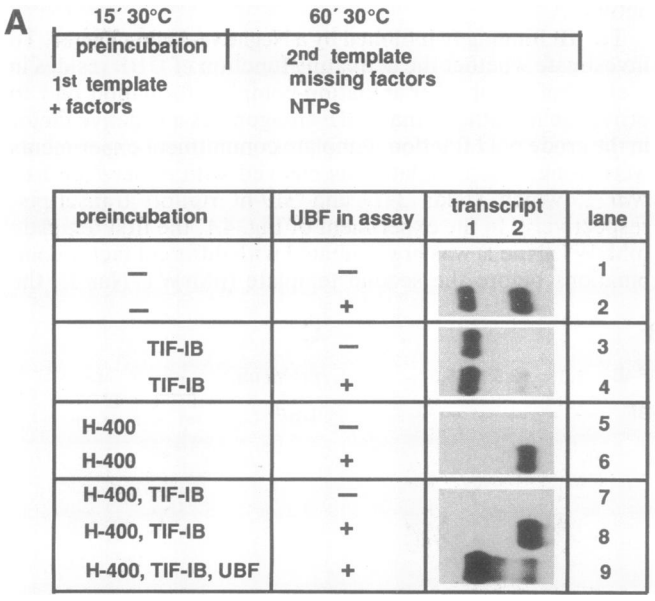


FIG. 3. Different responses of different pol I preparations to UBF-mediated transcription stimulation. The indicated amounts of pMrWT/Nde I were transcribed with or without 5 ng of UBF. (*A*) pol I fraction that had been fractionated by chromatography on DEAE-Sepharose and heparin-Ultrogel (H-400 fraction). (*B*) H-400 fraction that was further purified by chromatography on S-Sepharose and Mono Q columns.

missing factors, and the nucleoside triphosphates were added. As expected, both templates were transcribed and activated by UBF with the same efficiency when preincubation was omitted (lanes 1 and 2). Preincubation of the first template with TIF-IB (lanes 3 and 4) resulted in the preferential transcription of this DNA, even without UBF. This finding supports our previous notion that template commitment is brought about by TIF-IB alone and does not require UBF or other factors (1). However, when the preincubations were done with the H-400 fraction alone (lanes 5 and 6), UBF-mediated transcription exclusively occurred at the second template. Transcription from the first template was completely suppressed, indicating that the repressor remained stably bound to the template and prevented transcription complex formation. In contrast, preincubation of the first template with the H-400 fraction and TIF-IB before addition of the second template and the missing components differently affected transcription from either template (lanes 7–9). Surprisingly, addition of UBF after the preincubation period exclusively stimulated transcription from the second template (lane 8), whereas transcription from the first template compared to the control reaction without UBF (lane 7). However, when UBF was present during the preincubation reaction, the first template showed a preferential transcriptional commitment (lane 9). Again, this result indicates that without UBF the first template is repressed by a DNA-binding protein in the H-400 fraction. Apparently this inhibitory factor is present in higher amounts than TIF-IB or exerts a higher affinity for the rDNA promoter than TIF-IB does. Therefore, with the inhibitor, TIF-IB does not bind to the promoter, and the first template is not transcribed. However, when a second template and UBF were added after the preincubation period, TIF-IB promoter complexes were formed, and transcription from the second template occurred (lane 8).

A slightly different result was obtained when TIF-IB (or H-400) bound to the rDNA promoter was challenged with the H-400 fraction (or TIF-IB, respectively) before the second template, the missing factors, and the nucleotides were added. The rationale of this experiment was to ascertain how stably TIF-IB and the putative repressor bind to the rDNA promoter and how UBF affects this DNA–protein interaction. The result is shown in Fig. 4*B*. Lanes 1 and 2 are controls showing that preincubation of the first template with either TIF-IB or the H-400 fraction before adding the second template and the other factors results in the preferential transcription of the first or second template, respectively (comparable to lanes 4 and 6 in Fig. 4*A*). Reaction 3 is identical to reaction 1, except that after the first preincubation period the H-400 fraction was added. Incubation was continued for another 15 min before the assay was complemented with the remaining factors, the second template, and the nucleotides; both templates were then transcribed, indicating that during the second preincubation period in the presence of H-400 fraction a considerable portion of TIF-IB has been removed from the template. On the other hand, in



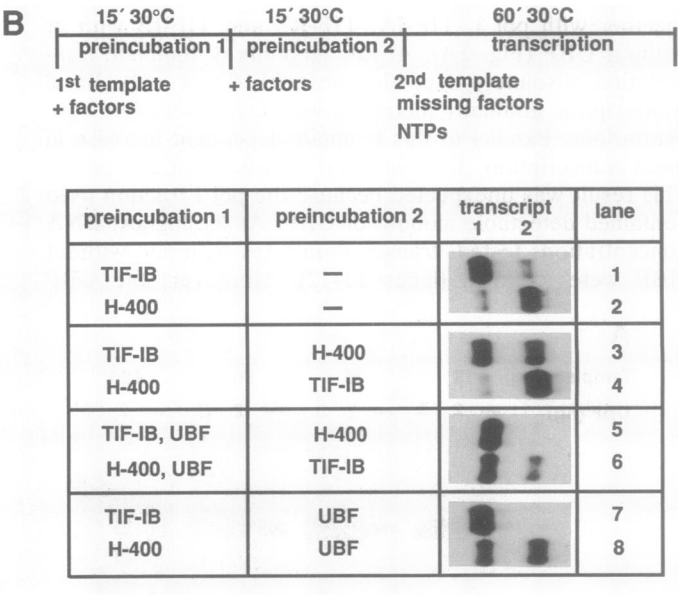


FIG. 4. Template commitment assays reveal a negatively acting rDNA-binding protein in the H-400 fraction. (*A*) Transcription reactions contained 5 ng each of pMrWT/Nde I (template 1) and pMrWT/Nar I (template 2). The first template was preincubated for 15 min at 30°C with the individual transcription factors. Then the second template, the nucleotides, and the missing factors were added; incubation was continued for another 60 min. Lanes 1 and 2 show control reactions without preincubation. (*B*) The first template (pMrWT/Nde I) was preincubated with the protein fraction(s) listed in the first column before the factors indicated in the second column were added, and incubation was continued for 15 min. Transcription was started by adding the second template, the missing proteins (including 5 ng of UBF), and the nucleotides. The first two lanes show control reactions where template 2 was added after the first preincubation period and allowed to compete for bound proteins for 15 min before transcription was started.

the reverse experiment—i.e., challenging prebound inhibitor with TIF-IB—transcription from the first template was not increased (lane 4), indicating that the putative repressor binds more strongly to the rDNA promoter than TIF-IB does. A different result was obtained when preincubation of the first template with either TIF-IB or H-400 included UBF. In this case TIF-IB binding was not affected by subsequent challenge with H-400 (lane 5), and the H-400-mediated transcriptional repression was relieved (lane 6). Similar effects were observed when UBF was present during the second incubation (lanes 7 and 8). This result shows that lacking UBF, the repressor binds more tightly than TIF-IB to the promoter and, therefore, inhibits transcription. UBF, on the other hand, appears to differently affect binding of TIF-IB and the negatively acting factor: it stabilizes TIF-IB and lowers repressor–DNA interactions.

UBF Counteracts Histone H1-Mediated Repression of Transcription. The preincubation and template commitment experiments strongly suggest the existence of a yet-unknown protein that competes with TIF-IB for binding to the promoter. Because the nature of this inhibitor is still unknown, we searched for a convenient system in which the antirepression effect of UBF could be investigated. Recent studies on the function of RNA polymerase II transactivators suggested that interactions between certain activator proteins and TFIID could prevent repression of transcription *in vitro* by histone H1 (8). We have shown that stable transcription-complex formation at the murine rDNA promoter was inhibited when the template DNA was preincubated with histones (9). Thus histone H1 bound to naked DNA appeared to be a reasonable model for a transcriptionally repressed rDNA promoter. To examine the ability of UBF to overcome the repression of transcription by purified histone H1, we used the more purified pol I (Fig. 3*B*), which at the template concentrations used (30 ng) was not stimulated by UBF. In the absence of UBF transcription progressively decreased after addition of histone H1 (Fig. 5, lanes 3, 5, 7). In the presence of UBF this H1-mediated repression was not observed (lanes 4, 6, 8). Hence, the increase in UBF-mediated transcription activity is not a true activation of transcription but rather the result of antirepression under conditions of repressed basal transcription.

DISCUSSION

Assembly of productive transcription-initiation complexes at the mouse rDNA promoter involves the cooperative action of two DNA-binding proteins, TIF-IB and UBF. UBF has been shown to stabilize TIF-IB binding to the promoter and to stimulate transcription initiation (1, 5, 7). It was concluded that UBF is required for transcription-complex assembly and

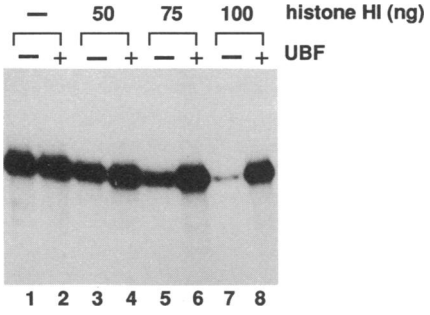


FIG. 5. UBF counteracts histone H1-mediated inhibition of basal transcription. Template DNA (30 ng) was transcribed in the reconstituted system containing 6 μl of pol I (Mono Q fraction), 2 μl of TIF-IB, 4 μl of TIF-IA/TIF-IC, and various amounts of histone H1. Transcriptions were done either without (lanes 1, 3, 5, and 7) or with (lanes 2, 4, 6, and 8) 5 ng of UBF.

is indispensable for transcription initiation (5–7). In this communication we present experimental evidence indicating that, at least in the mouse system, this interpretation should be modified. The degree of UBF-directed transcription stimulation was highly variable, depending either on the purity of the transcription factors used or on the concentration of template DNA (10). We found that increased amounts of TIF-IB or preincubation of the template with TIF-IB significantly raised basal transcription and reduced the UBF-induced transcription. The most puzzling observation, however, was the different response of various pol I preparations. Paradoxically, less pure fractions, which even contained detectable amounts of UBF, depended more on UBF than did highly purified fractions. A polymerase preparation fractionated by only two chromatographic steps was activated >50-fold, whereas under identical conditions a preparation purified by two more steps was stimulated only 5-fold. This result may be interpreted to mean either that we have removed an activator that mediates the UBF effect or that we have removed a negative factor that is counteracted by UBF.

Our results show that the second alternative is likely to be true, although we cannot exclude the possibility that, in addition to a negative component, we have also removed a positive-acting factor from pol I during fractionation. We postulate the existence of a yet-unknown cellular factor that antagonizes TIF-IB. This inhibitory factor appears to be present in crude pol I preparations and represses transcription by binding to the rDNA promoter, thus preventing formation of a productive preinitiation complex. Both TIF-IB and the inhibitor interact in a mutually exclusive way with the rDNA promoter, but apparently the repressor has a higher binding affinity than TIF-IB. Therefore, in the absence of UBF, the promoter is blocked, and practically no transcription is seen. This repression may be relieved either (*i*) by increasing the amount of template DNA that allows the inhibitor and TIF-IB to distribute on different DNA molecules, (*ii*) by preincubating TIF-IB with the template, or (*iii*) by addition of UBF. In agreement with previous investigations, UBF very likely increases the affinity of TIF-IB to its target sequence and, therefore, alleviates the effect of the putative repressor. In addition, UBF appears to destabilize the interaction between the repressor and the rDNA promoter. Whether or not this destabilization is from a direct physical association between UBF and the repressor remains to be investigated.

For transcription-complex formation, UBF may be required transiently and be dispensable once the inhibitory effect is overcome by formation of a binary TIF-IB–DNA complex. This model implies that UBF is an essential factor *in vivo* but is not absolutely required for transcription *in vitro*. Indeed, with highly purified factors, we always observe transcription without UBF. This basal transcription is only moderately stimulated by UBF (3- to 5-fold). *In vitro* UBF is required only if TIF-IB binding is repressed by a protein that interacts with the same or overlapping target sequence. Therefore, strong stimulation by UBF is seen with fractions that still contain this inhibitor. We postulate that the polymerase used contains this inhibitor and that UBF exerts a dual function: it weakens binding of the repressor to the promoter and interacts with TIF-IB to create a TIF-IB-containing, nonrepressible preinitiation complex that can promote multiple rounds of initiation by pol I.

At present we are still ignorant of the nature of the DNA-binding repressor protein and its mode of action. We could simulate UBF-directed transcription activation in a model system, showing that UBF counteracts histone H1-mediated repression of basal transcription. Similarly, recent studies about the function of RNA polymerase II transactivators suggested that interactions between certain activator proteins and TFIID prevent repression of transcription *in*

vitro by histone H1 or assembled nucleosomes (8). These studies, as well as the results reported here, suggest that transcriptional antirepression is probably a general property of several promoter- and enhancer-binding factors and that activation and antirepression are distinct functions of sequence-specific activators. Activator proteins (including UBF), therefore, appear to function in two ways—by facilitating the breakdown of structural impediments to transcription-complex formation and by participating in the formation of active complexes. This dual role could reflect a single mode of action. Both the relief of inhibition and stimulation of initiation-complex formation could be mediated by a direct contact between UBF and either the repressor or TIF-IB. As a result, the repressor is dislodged from the template, and TIF-IB binding is stabilized.

The existence of a protein that binds to the rDNA promoter and prevents binding of TIF-IB and the subsequent formation of a preinitiation complex would offer an excellent switch mechanism for control of ribosomal gene transcription. It has been suggested that rDNA transcription is regulated at two relatively independent levels (11). The first level involves the establishment of stable transcription-initiation complexes, which in turn reflect the number of active genes. This level of regulation is probably mediated by a balanced antagonism between the action of TIF-IB, UBF, and the putative inhibitor. This coarse control may be the major determinant for the fraction of active transcription units during cellular development and differentiation. The second level of regulation involves a regulatory factor (TIF-IA) that fluctuates in amount or activity according to the physiological state of the cells (12, 13). The level of TIF-IA finally determines the actual amount of transcription that occurs on open, potentially active genes.

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