Functional Cooperativity Between Transcription Factors UBF1 and SL1 Mediates Human Ribosomal RNA Synthesis

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The human ribosomal RNA promoter contains two distinct control elements (UCE and core) both of which are recognized by the sequence-specific DNA binding protein UBF1, which has now been purified to apparent homogeneity. The purified factor activates RNA polymerase I (RNA pol I) transcription through direct interactions with either control element. A second RNA pol I transcription factor, designated SL1, participates in the promoter recognition process and is required to reconstitute transcription in vitro. Although SL1 alone has no sequence-specific DNA binding activity, deoxyribonuclease I footprinting experiments reveal that a cooperative interaction between UBF1 and SL1 leads to the formation of a new protein-DNA complex at the UCE and core elements. In vitro transcription experiments indicate that formation of the UBF1-SL1 complex is vital for transcriptional activation by UBF1. Thus, protein-protein interactions between UBF1 and SL1 are required for targeting of SL1 to cis-control sequences of the promoter.

INITIATION OF TRANSCRIPTION IN EUKARYOTIC ORGANISMS IS a complex process requiring one of three distinct RNA polymerases and a number of auxiliary transcription factors. Biochemical studies of the transcription reaction suggest that both specific protein-DNA and protein-protein interactions are required for accurate and regulated initiation of transcription (*1*). The purification and characterization of transcription factors that bind DNA sequence specifically have added significantly to our understanding of protein-DNA contacts mediating transcription (*2*). In contrast, much less is known about the associations between different proteins required for efficient transcription. Thus, it is of interest to study transcription factors that have no apparent DNA binding activity but are nevertheless required for promoter specificity (*3, 4*). It is likely that such factors interact either with RNA polymerase or with factors already bound to the promoter. Transcription of human ribosomal RNA (rRNA) provides a system for investigating the interactions between transcription factors because an essential factor (SL1), which has no apparent sequence-specific DNA binding activity, is responsible for conferring promoter selectivity on RNA pol I (*3, 4*). Although the mechanism of SL1-mediated promoter recognition which we postulate is not yet known, our data suggest this recognition process must involve specific protein-protein interactions.

Genetic analysis of the human rRNA promoter both in vivo and in vitro defined two distinct cis-regulatory DNA sequences required for efficient initiation by RNA pol I (*5, 6*). The core promoter element extends from nucleotides +20 to −45, overlaps the start of transcription (+1), and contains sequences that are essential for specific initiation. The upstream control element (UCE) spans the region from −107 to −186 and enhances initiation by up to a factor of 15 in vitro and 100 in vivo. The activity of the UCE is sensitive to changes in both orientation and position relative to the core element, suggesting that these two cis-control sequences operate in concert to promote efficient transcription by RNA pol I (*6*). The bipartite configuration of the human rRNA promoter is also observed in the rRNA promoters of other organisms, including *Xenopus* and mouse (*7*). Despite the similarity in overall structure, different mammalian RNA pol I promoters share little sequence homology and are not recognized by the transcription machinery of a heterologous species unless they are closely related (for example, human and monkey) (*8*). This species-specific promoter recognition appears to be mediated by one or more auxiliary transcription factors rather than by RNA pol I, which is functionally conserved. For example, although a mouse extract cannot ordinarily recognize the human rRNA promoter, the addition of a human factor, SL1, to the mouse extract allows the efficient transcription of a human template (*3*).

SL1 has been isolated and shown to be essential for human RNA pol I transcriptional initiation in vitro (*3*). To investigate the mechanism of SL1-dependent transcriptional initiation, we have characterized the protein-DNA interactions of SL1 and other cellular factors with the rRNA promoter. Although SL1 exhibited no sequence-specific DNA recognition properties, a DNA binding activity that recognizes the rRNA promoter was identified in a partially purified RNA pol I fraction (*9*). Deoxyribonuclease (DNase) I footprinting experiments revealed that this activity, designated upstream binding factor 1 (UBF1), protects sequences overlapping a portion of the UCE. To address the function of this binding protein during transcription of the rRNA promoter, we have separated UBF1 activity from RNA pol I as well as other contaminating proteins. Purified UBF1 was used to investigate its role in the formation of a novel SL1-dependent complex with the promoter. In addition, we have studied the effect of UBF1 on initiation of transcription in reconstituted transcription reactions.

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Finally, we have analyzed the mechanism of SL1-dependent transcriptional activation through its interactions with the UCE. Our findings suggest that protein-protein interactions between two transcription factors, UBF1 and SL1, mediate the function of the UCE as well as the core element.

Purification of UBF1 by DNA affinity chromatography. Both conventional and sequence-specific DNA affinity chromatography were used to purify UBF1 from HeLa cells. First, fractionation of a nuclear extract by heparin-agarose chromatography separated UBF1 from SL1 and the bulk of the RNA pol I activity. A Bio-Rex 70 column removed both residual RNA pol I activity as well as various nonspecific DNA binding activities from UBF1. The final step in the purification of UBF1 involved sequence specific DNA affinity chromatography (*10*); UBF1 was analyzed by SDS-gel electrophoresis at various stages during the purification (Fig. 1A). Two polypeptides of 94 and 97 kD were highly enriched by the specific DNA affinity resin and the most purified fractions contained only these two proteins (unless otherwise indicated this material was used for all subsequent experiments). We estimate that UBF1 was purified by a factor of at least 10,000 by this procedure, and typically 5 to 10 μg was obtained from 60 g of HeLa cells.

To identify the polypeptides responsible for the DNA binding activity of UBF1, we subjected DNA affinity-purified UBF1 (first pass) to SDS-gel electrophoresis and cut the resulting gel into several fragments (Fig. 1B). The proteins contained in each of the fragments were eluted, renatured, and assayed for sequence-specific DNA binding activity (Fig. 1C). Only the fragment containing the 94- and 97-kD proteins (fraction 4, Fig. 1, B and C) had UBF1 binding activity. Partial cleavage of the separated 94- and 97-kD polypeptides with V8 protease revealed that the two proteins are closely related in their primary structure (*11*). Further fractionation of purified UBF1 has confirmed that the 97-kD polypeptide alone is sufficient for UBF1 binding (*12*). Thus, UBF1 is composed of two closely related polypeptides that are responsible for the UBF1 DNA binding activity.

UBF1 interactions with the promoter and SL1. DNase I footprinting of the pol I promoter reveals that purified UBF1 protects a region between −75 and −114 (Fig. 2), overlapping the 3′ portion of the UCE. Enhanced DNase I cleavage sites are observed on both the coding and noncoding strands at positions −96 and −95, respectively. Previous binding studies with crude preparations of UBF1 indicated that, in addition to the DNase I–protected region overlapping the UCE, a weaker interaction with core sequences resulted in an enhanced cleavage at position −21 (*9*). However, in these earlier studies we could not determine whether the enhanced cleavage site in the core element was due to UBF1 or a contaminant in the partially purified samples. Several observations now firmly establish that UBF1 also interacts with the core element. First, the enhanced cleavage at −21 is observed even with the most purified UBF1 samples. The core binding activity comigrates with UBF1 binding activity at the UCE throughout the purification procedure, including SDS-gel purification and renaturation. Second, the core element contains a region of significant sequence homology (85%) with an element in the UCE important for UBF1 binding (see below). These regions of homology straddle the enhanced cleavage site observed in both the UCE and core binding domains. Finally, an affinity resin prepared from oligomers derived from core sequences purifies the same 94- and 97-kD polypeptides identified as UBF1 (*13*). Thus UBF1 binds specifically to both of the previously identified human rRNA promoter elements although with differing affinities.

We have shown previously that the addition of SL1 to crude

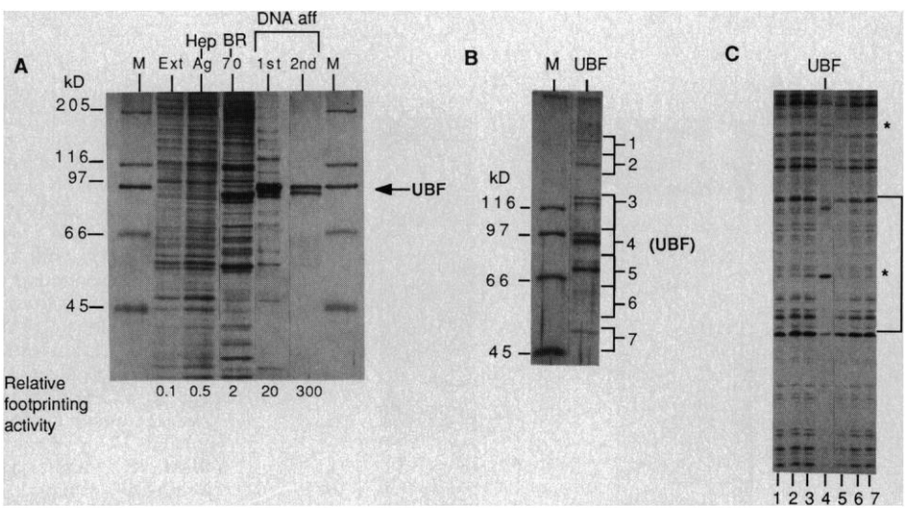

Fig. 1. Purification of UBF1. (**A**) Nuclear extract from 60 g of HeLa cells was prepared by the procedure of Dignam *et al.* (*23*), except that NaCl was replaced with KCl. The resulting extract was brought to 60 percent saturation with ammonium sulfate and centrifuged at 35,000*g* for 20 minutes; the pellet was resuspended in TM buffer (50 m*M* tris-HCl, *p*H 7.9, containing 12.5 m*M* $MgCl_2$, 1 m*M* EDTA, 1 m*M* dithiothreitol (DTT), and 20 percent glycerol) to a final protein concentration of about 20 mg/ml and dialyzed against TM buffer containing 0.1*M* KCl. The soluble protein extract was applied to a heparin agarose column (90 ml) (*24*) equilibrated with TM buffer containing 0.1*M* KCl. The column was washed with TM containing 0.2*M* KCl, and protein was eluted with a linear gradient (800 ml) from 0.2*M* to 1.0*M* KCl. Fractions were collected and assayed for UBF1 activity, and the peak of activity was pooled (eluted at approximately 0.45*M* KCl). This material was dialyzed against TM, 0.1*M* KCl and applied to a Bio-Rex 70 column (Bio-Rad, 15 ml) equilibrated against the same buffer. The column was washed with TM, 0.3*M* KCl, and the protein was eluted with a linear salt gradient between 0.3*M* KCl and 1.0*M* KCl. The fractions containing UBF1 were pooled (eluted at approximately 0.6*M* KCl), and dialyzed against TM, 0.1*M* KCl, 0.1 percent Nonidet P-40 (NP-40). This material was mixed with sonicated salmon sperm DNA (20 μg per milligram of protein, Bio-Rex pool) and poly[d(A-T)·d(A-T)] (400 μg per milligram of protein, Bio-Rex pool) and held for 30 minutes at 4°C. It was then applied to a DNA affinity column (*10*). The UBF1 affinity resin was prepared with two oligonucleotides derived from the UCE of sequence 5′-CAGGT GTCCG TGTCG CGCGT CGCCT GGGCC GGCGG CG-3′ and 5′-ACCTG CGCCG CCGGC CCAGG CGACG CGCGA CACGG AC-3′. After the column was washed with TM, 0.1*M* KCl, 0.1% NP-40, bound protein was eluted with TM, 0.6*M* KCl, 0.1% NP-40. The eluate was then diluted to 0.1*M* KCl with TM, 0.1% NP-40 and passed over the affinity resin again, but with half the original DNA competitor. The indicated pools of UBF1 were precipitated with trichloroacetic acid and subjected to SDS-polyacrylamide electrophoresis (*25*) and silver-stained. M, markers; Ext, nuclear extract; Hep-Ag, Heparin agarose pool; BR-70, Bio-Rex 70 pool; and DNA aff, DNA affinity column pools (1st, first pass; 2nd, second pass). The approximate relative footprinting activity is indicated at the bottom of each lane. (**B**) Renaturation of UBF1 binding activity. Approximately 1 μg of first pass affinity-purified UBF1 was prepared for electrophoresis (*26*). The bracketed regions of the SDS-polyacrylamide gel were excised and the protein contained in the slices was eluted and renatured (*26*). (**C**) The resulting renatured protein samples were assayed for UBF1 DNA binding activity by DNase I protection of the non-coding strand of the rRNA promoter. The protected region is bracketed and asterisks mark the characteristic enhanced cleavages (see Fig. 2A). The numbers at the bottom of the panel indicate the bracketed region from which the proteins were eluted.

RNA pol I fractions containing UBF1 binding activity resulted in the enhancement of the normal UBF1 footprint as well as the formation of new protected regions (*9*). To establish unambiguously the requirements for UBF1 in the formation of this SL1-dependent complex, we performed DNase I protection assays with purified UBF1 in the presence and absence of SL1. Comparison of the results obtained with purified UBF1 (Fig. 3B) and those obtained with crude RNA pol I preparations used previously (Fig. 3A) indicates that identical footprints were observed over the UCE. DNase I protection of the UBF1 binding site is dramatically enhanced in the presence of SL1 (site A) and a new protected region is observed between −115 and −165 (site B). It is not yet clear whether SL1 makes direct contact with DNA in the presence of UBF1 or whether the recognition properties of UBF1 are altered in the presence of SL1, resulting in new protected sequences. Addition of RNA pol I depleted of UBF1 and SL1 has no effect on the protected sequences or the formation of the SL1-dependent complex (*14*). In addition to interactions at the UCE, SL1 also improves binding of UBF1 to the core element. In the presence of SL1, the enhanced cleavage at −21 becomes more prominent and, with purified UBF1, addition of SL1 results in protection of sequences between −21 and the start site of transcription at +1 (Fig. 3B, lanes 4 and 5). Thus, the addition of SL1 to purified UBF1 results in the formation of new protein-DNA contacts at the core and the UCE of the rRNA promoter.

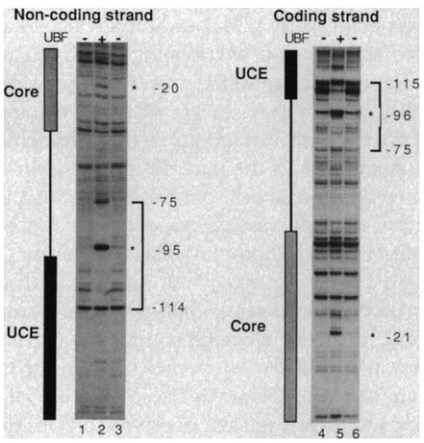

Fig. 2. DNase I footprint analysis of the purified UBF1 protein. DNase I protection experiments were carried out as described (*9*), except that competitor DNA was not added. A 279-bp fragment (−199 to +78, labeled at −199) of the human rRNA promoter was used to assay the noncoding strand (lanes 1 to 3). A 525-bp fragment (−500 to +24, labeled at +24) of the human rRNA promoter was used to assay the coding strand (lanes 4 to 6). Lanes 1, 3, 4, and 6 had no protein added to the DNase I treatment and lanes 2 and 5 had 5 ng of purified UBF1 added during DNase I treatment. Lanes 1 to 3 were run on a 6 percent polyacrylamide gel and lanes 4 to 6 were run on an 8 percent polyacrylamide gel. The position of promoter elements relative to the DNase I ladder is indicated to the left of each footprint. Bracketed regions indicate protected regions and asterisks indicate enhanced cleavages. Nucleotide positions relative to the start site were determined from the corresponding Maxam and Gilbert sequencing ladders for each probe (*27*).

Activation of transcription by UBF1 requires sequences outside of the UBF1 binding site. A critical issue remaining concerned the ability of UBF1 to act as a transcription initiation factor for RNA pol I. To address this point directly, we assayed UBF1 activation of ribosomal DNA (rDNA) transcription by adding purified protein to a reconstituted transcription reaction depleted of UBF1 binding activity. Two DNA templates were added to each reaction, one containing the entire promoter and a second with a deletion of the UCE, which therefore lacks the upstream UBF1 binding site. As expected, no transcription was observed from either of the templates in the absence of SL1, an indication that UBF1 does not complement SL1 activity (Fig. 4, lane 1). Transcription in the absence of added UBF1 results in the same low basal level of activity from both the full-length and the truncated templates (*15*) (Fig. 4, lane 2). Addition of increasing amounts of purified UBF1 to the transcription reaction dramatically enhances initiation from

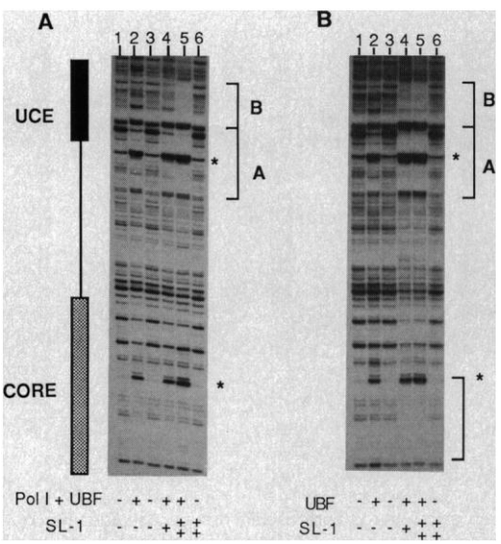

Fig. 3. Interaction of purified UBF1 and SL1 with the rRNA promoter. Footprinting reactions were carried out as described in Fig. 2. A 525-bp fragment end-labeled at +24 was used as probe (see Fig. 2). Binding reactions contained the following, as indicated below each lane: pol I, 30 μg of partially purified RNA pol I; SL1, either 0.3 (+) or 0.9 μg (++) of concentrated human SL1; UBF1, 5 ng of purified UBF1. Lanes including SL1 fractions received 10 ng of competitor DNA poly[d(A-T)·d(A-T)]. Both partially purified RNA pol I and concentrated SL1 were prepared as described (*9*). The relative position of promoter elements to the DNase I ladder is indicated to the left of each panel, and protected regions and enhanced cleavage are indicated by brackets and asterisks, respectively. Site A extends from −75 to −114 and site B from −115 to −165.

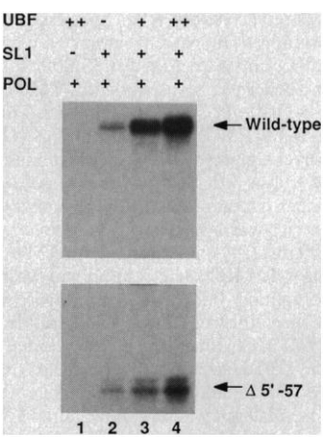

Fig. 4. Activation of RNA pol I initiation by purified UBF1. Reconstituted transcription reactions were used to transcribe an equimolar ratio of a pseudo wild-type template and a 5′ deletion to −57 (total of 100 ng). RNA Pol I was prepared as described in (*9*) with the following changes. The starting material was the void fraction from a Sephacryl-300 (Pharmacia) gel filtration column of a nuclear extract prepared according to (*23*) instead of whole cell extract. Pooled material after passage through the heparin agarose column was dialyzed against TM, 0.1*M* KCl, and loaded onto a Mono-Q column (Pharmacia) equilibrated against the same buffer (this replaces the DEAE column in the previous procedure). Protein was eluted with a salt gradient from 0.1 to 0.5*M* KCl. The active fractions were pooled and dialyzed against TM, 0.1*M* KCl. This material contained no detectable UBF1 binding activity. Each reaction contained 10 μg of RNA pol I. Reactions 2, 3, and 4 contained 1 ng of SL1. Reactions 1, 3, and 4 contained 30, 10, and 30 ng of purified UBF1, respectively. Transcription reactions were performed and RNA was prepared as described except that reactions were performed at 110 m*M* KCl (*9*). S1 nuclease analysis was carried out on RNA's annealed to ^{32}P-labeled single-stranded probes. 5′-Labeled oligonucleotides were made identical to the coding strand of the rRNA promoter between −20 and +40 of the wild-type promoter or between −20 and +80 of the coding strand of the pseudo wild-type template. The pseudo wild-type template was constructed by inserting a 200-bp fragment of pUC 13 at the Sac I site in LSM +10/+20 (*6*) and transcribes with efficiency equal to that of a normal wild-type promoter.

the wild-type template (15-fold), while the template lacking the UCE, but containing an intact core element, was activated less efficiently (only fourfold; Fig. 4, lane 4). The effect of the UCE observed in the fully reconstituted transcription reaction (including UBF1) is consistent with the effects of the UCE observed in crude extracts (*6, 16*). Two conclusions can be made from these results. First, UBF1 is an RNA pol I transcription factor and activates transcription either in the presence or the absence of the UCE, providing additional evidence for a role of UBF1 in the core element. Second, UBF1 appears to be necessary for the UCE to activate transcription in vitro, as the level of initiation in the absence of added UBF1 is not affected by the UCE.

To define the cis-control sequences responsible for UBF1 activation of transcription and correlate its binding to transcriptional activation, transcription assays were performed in the presence or absence of UBF1 with various mutants of the rRNA promoter. As expected, a substitution mutant (CPM11) that prevents the binding of UBF1 to the UCE was activated to a similar low level as a deletion of the upstream sequences (Fig. 5A, lanes 13 and 14). Interestingly, mutations that do not affect binding of UBF1 to the rDNA promoter but instead prevent the formation of the SL1-dependent complex also show reduced activation by UBF1. For example, a linker scanner mutant, LSM−149/−131, prevents the formation of the UBF1/SL1 complex but does not alter the binding of UBF1 alone (*9*) (Fig. 5B). When LSM −149/−131 is used to direct transcription we observe significantly reduced levels of activation by UBF1. This reduced UBF1 activation is equivalent to that observed with a mutant with the UCE deleted (compare Fig. 5A, lanes 10 and 14). Similar results were obtained with the mutant LSM −108/−113 and a 5′ deletion to −131 (Δ5′/−131) which also prevent the formation of the UBF1/SL1 complex but do not interfere with UBF1 binding (Fig. 5A, lanes 11 and 12, and Fig. 5B). In the absence of added UBF1 there is no significant difference in the basal level transcription observed from any of the UCE mutants. These results indicate that the binding of UBF1 at the UCE, although necessary, is not sufficient to allow UBF1 activation of transcription. Instead, the correlation between DNA binding and transcriptional activation studies presented here strongly suggests the interaction between UBF1 and SL1 is essential for transcriptional activation by the UCE in vitro.

Because purified UBF1 also binds to and activates transcription from the core element, mutants in these sequences were tested for activation of transcription by UBF1 (Fig. 5, C and D). Interestingly, mutants LSM −33/−24 and LSM −32/−24 show significant differences in their response to UBF1, although they differ in sequence at only two base pairs. LSM −33/−24 exhibits normal

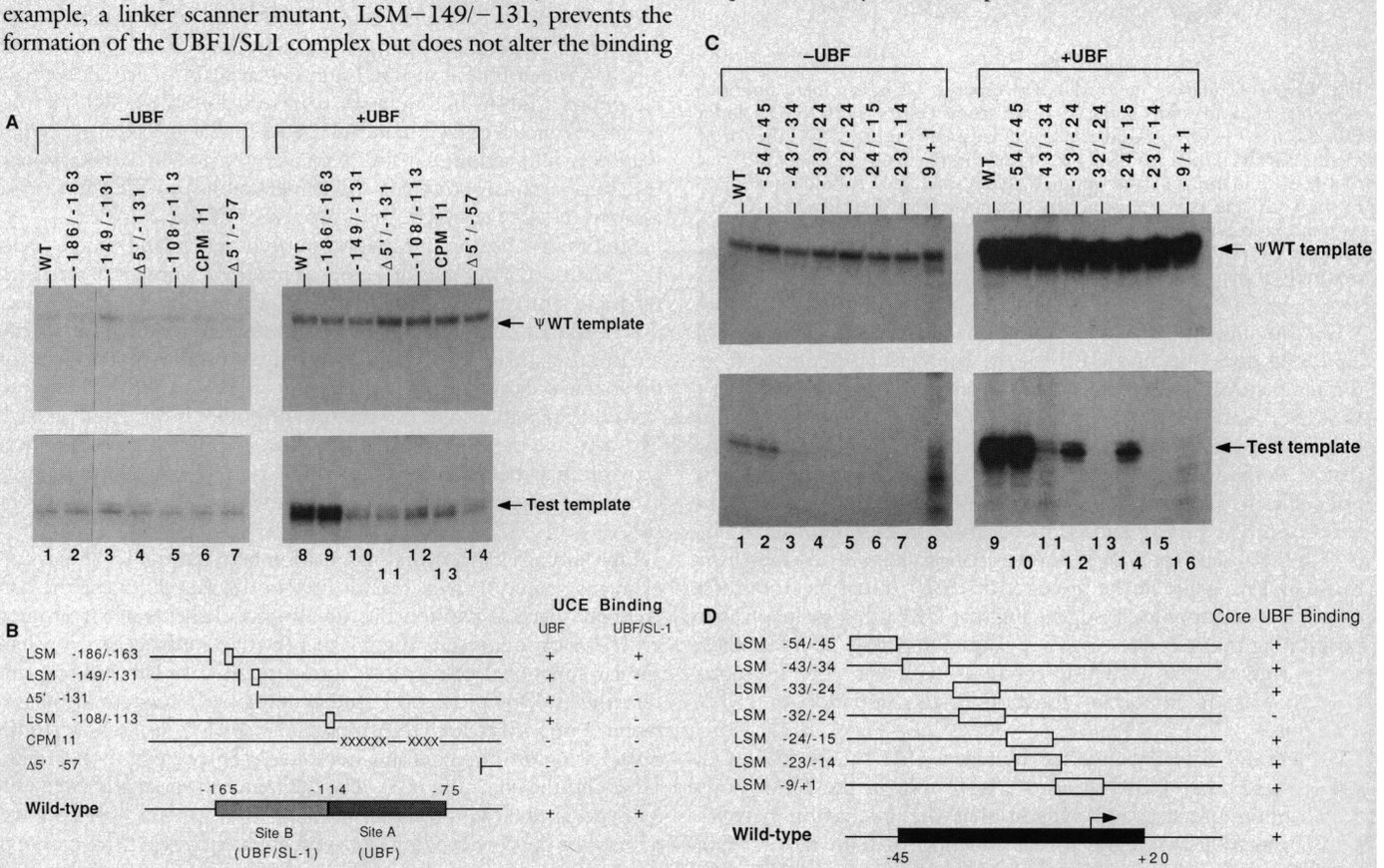

Fig. 5. Effect of mutants in the rRNA promoter on UBF1 activation of transcription. In vitro reconstituted transcription reactions were assayed by S1 analysis as described for Fig. 4. All reactions contained 10 μg of RNA pol I (prepared as described for Fig. 4), and 1 ng of SL1. Arrows indicate the expected size for the protected fragments from each promoter. The test template in each reaction is shown at the top of each lane. (**A**) Effect of upstream mutations. Each reaction contained a threefold molar excess of test template to pseudo wild-type template (total of 50 ng). UBF1 (+) reactions received 15 ng of purified UBF1. UBF1 (−) lanes were exposed three times longer than the UBF1 (+) lanes. (**B**) Binding properties of mutants used in part (A) transcription experiments. The wild-type diagram shows the sequences protected by either UBF1 alone (site A) or the combination of UBF1 and SL1 (sites A and B). Lines indicate wild-type sequence, boxes indicate linker sequences, X indicates sequences altered by site-directed mutagensis, and spaces are deleted sequences; the CPM 11 mutant was constructed as described (*28*). The ability of UBF1 to bind to the upstream sequences of a template is shown in the column marked UBF; the ability of a template to allow the formation of the UBF1/SL1 complex is shown in the column headed UBF/SL1. (**C**) Effect of mutations in the core element on UBF1 activation of transcription. Each reaction contained an equimolar ratio of test template to pseudo wild-type template (total of 50 ng). UBF1 (+) reactions received 15 ng of purified UBF1. (**D**) Binding properties of mutants used in (C). The black box in the wild-type construct indicates the core promoter element. The arrow indicates the start site of transcription. Lines indicate wild-type sequences, white boxes indicate linker sequences. The ability of UBF1 to bind to the mutant core promoter element is indicated below the column headed core UBF binding.

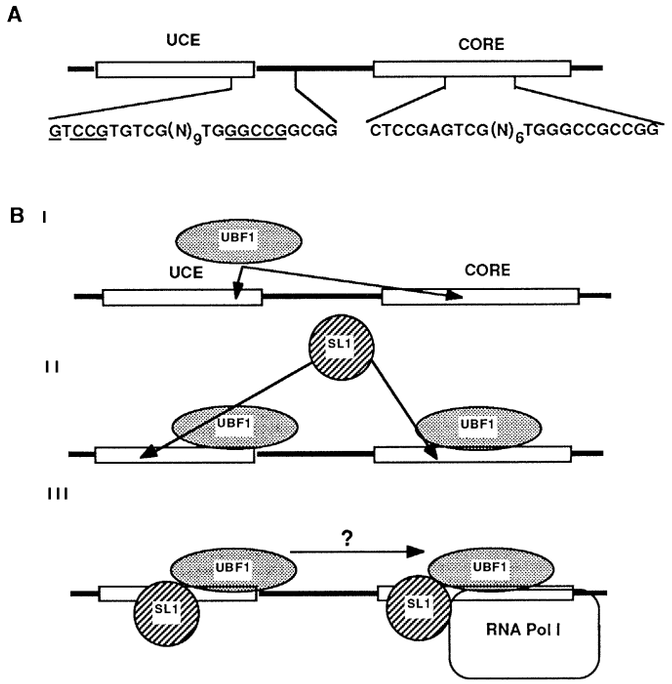

Fig. 6. (**A**) Sequences conserved between the UCE and core promoter elements. The end points of the upstream sequences are −110 to −81 and in the core are −34 to −7. Underlined sequences are protected by UBF1 from methylation by dimethyl sulfate (**B**) Model for the involvement of UBF1 and SL1 in the initiation of transcription by RNA pol I. Step I: Binding of UBF1 to the UCE and the core promoter elements. Step II: Interaction of SL1 with the template and bound UBF1. Step III: Recognition of the UBF1/SL1 complex either directly or indirectly by RNA pol I, leading to productive initiation of transcription.

UBF1 binding and strong transcriptional activation, whereas LSM −32/−24 does not bind UBF1 at the core and transcription from this template cannot be detected in the presence or absence of UBF1 (Fig. 5C, lanes 12 and 13, and Fig. 5D). The remaining mutants bind UBF1 efficiently but are transcriptionally impaired suggesting that, as in the UCE, the interaction of UBF1 with core sequences is not sufficient for activation of the promoter. Also of interest is the effect of UBF1 on the transcripts observed from mutant LSM −9/+1. In the absence of UBF1, novel initiation sites are observed from this template, while in the presence of UBF1 initiation from these cryptic sites is suppressed, suggesting that UBF1 may be involved in positioning the start site (Fig. 5C, lanes 8 and 16). Together these results suggest that UBF1 interactions with the core promoter element play an important function in the initiation of rRNA synthesis.

Functional interdependence between UBF1 and SL1. Our data indicate that UBF1 plays a crucial role in the RNA pol I transcription process, and suggest that the interaction between UBF1 and SL1 is integral to the function of both factors. Our previous studies indicated that promoter selectivity by RNA pol I requires SL1. However, we found no evidence that SL1 can bind specifically to rRNA promoter sequences (*3*). Thus, we postulated that another protein (or proteins) must be involved in targeting SL1 to the rRNA promoter. We now present several lines of evidence that UBF1 is responsible for coordinating SL1 function. DNase I footprinting experiments demonstrate that purified UBF1 is necessary and sufficient for SL1-dependent interactions at both the UCE and core elements, suggesting a protein-protein interaction between the two factors. In vitro transcription experiments indicate that UBF1 activates initiation through interactions with both the core and UCE binding sites. Moreover, analysis of UCE mutants indicates that UBF1 binding alone is not sufficient for transcriptional activation. Instead, our findings demonstrate that the UBF1-SL1 complex is required for UBF1 activation of transcription. Together these results suggest that the function of UBF1 is to localize SL1 to both the UCE and core promoter elements. Because SL1 is not yet a homogeneous preparation we cannot exclude the possibility that multiple polypeptides are required for full SL1 activity. However, the importance of the UBF1-SL1 complex for transcriptional activation suggests that the factor (or factors) required for complex formation is also important for promoter function.

In addition to revealing an interesting aspect of the mechanism of RNA pol I initiation, the interdependence of UBF1 and SL1 provides a biochemical model for the action of transcription factors that do not bind DNA alone but nevertheless confer specificity to the transcriptional machinery. Promoter elements that require both a DNA binding activity as well as a trans-activator or -repressor to modulate their function have been described (*17*). It is likely that some DNA-bound transcription factors act through protein-protein associations with either general transcription factors that do not bind DNA (for example, TF-IIB) (*4*) or RNA polymerase itself (*18*). The cooperative interaction between UBF1 and SL1 provides a clear example of promoter recognition by the interaction of two transcription factors. Several aspects of RNA pol I transcription should now allow a detailed study of its activation mechanisms. First, highly purified UBF1 and SL1 are now available. Second, we have developed a DNA binding assay to detect the UBF1-SL1 complex and have characterized mutants in the UCE that specifically interfere with this interaction. Finally, reconstituted in vitro transcription reactions responsive to UBF1-SL1 interactions provide a functional assay to dissect the biochemical properties of this complex.

Studies of RNA pol I initiation in both human and other species have identified a number of factors or fractions whose characteristics resemble a mixture of UBF1 and SL1. Protein fractions have been described that bind to the rRNA promoter, are required for specific initiation, and, in some cases, confer new promoter specificity on the extracts derived from a heterologous species (*19*). These fractions may possibly contain a mixture of both a UBF1- and an SL1-like activity, or in systems other than the human system, both activities may be performed by a single polypeptide. Further purification of these fractions should distinguish between these possibilities.

Our findings suggest that the relation between the UCE and core elements should be reevaluated. Previously, the UCE and the core were perceived as two functionally distinct elements of the promoter. However, our results suggest that the two control elements share similar functional properties. Both mutant template binding and dimethyl sulfate protection studies have been used to define sequences important for DNA binding by UBF1 (*14*). These studies reveal a region of significant homology (85 percent) between the UCE and the core (Fig. 6A). The difference in spacing between the half sites at the two promoter elements may explain UBF1's lower affinity for the core binding site. This apparently repetitive nature of cis-control sequences of the human rRNA promoter is also evident in other RNA pol I promoters. For example, the distant upstream regions of the *Xenopus* rRNA promoter contain multiple repeats of a 50-bp region homologous to the proximal promoter, and these sequences enhance the level of RNA pol I initiation (*20*). However, unlike the *Xenopus* RNA pol I upstream elements or many RNA pol II enhancers, the human UCE functions only within a restricted distance and orientation with respect to the core (*6*). Such spatial restrictions suggest that the UCE and core act in a cooperative manner, and it is possible that the role of the tandem UBF1 binding motifs is to coordinate the interplay between the promoter elements.

A model for promoter recognition by RNA pol I. Our current

model for promoter recognition by human RNA pol I is shown in Fig. 6B. The first step in transcriptional initiation is postulated to be binding of UBF1 at both the UCE and core. Next, SL1 interacts with the bound UBF1 at both sites. The resulting UBF1-SL1 complexes are then recognized by RNA pol I, leading to the initiation of transcription. Additional purification of RNA pol I results in preparations that show an increased requirement for UBF1 addition for specific initiation of transcription, supporting the important role of UBF1 suggested by the model (*14*). One prediction of this model is that both UBF1 and SL1 should be required for promoter recognition. However, we have demonstrated that SL1 alone is sufficient to confer human promoter specificity on a mouse extract (*3*). Consequently, we would predict that mouse cells must contain both an activity that recognizes the mouse UCE and core and a factor that can functionally replace human UBF1 and recognize the human promoter. Indeed, fractionation of mouse extracts reveals that a protein equivalent to human UBF1 in its binding and transcriptional activation properties is present in mouse cells, suggesting that SL1 reprograms the template specificity of a mouse extract in concert with this factor (*21*).

Despite the functional cooperativity between UBF1 and SL1, they appear to represent distinct classes of transcription factors. In addition to differences in DNA binding properties, UBF1 and SL1 also appear to activate transcription by distinct mechanisms. Whereas UBF1 must be added in quantities sufficient for complete binding of the template for full activation of transcription, our results indicate that SL1 is able to activate transcription when present in amounts considerably less than one molecule per template used (*3*). Unlike UBF1, SL1 exhibits a nonlinear response in transcriptional efficiency during titration experiments. Finally, the number of SL1 molecules per cell is estimated to be 100 to 400, whereas UBF1 appears to be present at approximately 50,000 copies per cell, compared to approximately 200 copies of the rRNA gene. The above results have led us to think that SL1 may act in a cyclic manner at the promoter, perhaps in a fashion analogous to prokaryotic sigma factors (*22*). However, unlike sigma factors, SL1 interacts with a sequence-specific DNA binding protein that coordinates its function at the promoter rather than binding to RNA polymerase itself. Through the use of purified UBF1 and isolation of the gene encoding UBF1 we are now in the position to determine how these apparently different transcription factors act together to promote RNA pol I initiation.

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12. Fractionation of 2.5 μg of affinity-purified UBF1 on a Mono-S ion exchange column resulted in fractions containing only the 97-kD polypeptide. When assayed for DNA binding by DNase I footprinting, these fractions protected regions indistinguishable from those protected by the affinity-purified UBF1 starting material (94- and 97-kD polypeptides together). No fractions were obtained in which the 94-kD polypeptide was completely separated from the 97-kD.
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15. Quantitation of transcripts by scintillation counting of gel slices as well as densitometric scanning of autoradiograms indicates that the same amount of transcript is observed from both templates in lane 2. Quantitation of lane 4 indicates that activation of the wild-type template increases 15-fold on addition of UBF while that of the deleted template increases only 4-fold.
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28. CPM 11 was constructed by replacing the sequence between −74 and −170 in the linker scanning mutant LSM −73/−74 (11) with six synthesized oligonucleotides of approximately 30 bp each. The oligonucleotides were the wild-type promoter sequence except between the regions of −110 and −100, and −87 and −80. The sequence of the construct was confirmed by DNA sequencing. The resulting template showed no binding of UBF1 at the upstream site.

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