Assembly of alternative multiprotein complexes directs rRNA promoter selectivity

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How can *trans*-activators with the same DNA binding specificity direct different transcriptional programs? The rRNA transcriptional apparatus offers a useful model system to address this question and to dissect the mechanisms that generate alternative transcription complexes. Here, we compare the mouse and human transcription factors that govern species-specific RNA polymerase I promoter recognition. We find that both human and mouse rRNA transcription is mediated by a specific multiprotein complex. One component of this complex is the DNA-binding transcription factor, UBF. Paradoxically, human and mouse UBF display identical DNA binding specificities even though transcription of rRNA is species specific. Promoter selectivity is conferred by a second essential factor, SL1, which, for humans, does not bind DNA independently but, instead, cooperates with UBF in the formation of high-affinity DNA-binding complexes. In contrast, mouse SL1 can selectively interact with DNA in the absence of UBF. Reconstituted transcription experiments establish that UBF and RNA polymerase I from the two species are functionally interchangeable, whereas mouse and human SL1 exhibit distinct DNA binding and transcription activities. Together, these results suggest a critical role for a specific multiprotein assembly in RNA polymerase I promoter recognition and reveal distinct mechanisms through which such complexes can generate functional diversity.

[*Key Words*: RNA pol I; rRNA transcription; species specificity; transcription factors; UBF; SL1]

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Transcriptional initiation in eukaryotic cells is a highly regulated process requiring the correct association of numerous proteins into a specific complex with RNA polymerase (Mitchell and Tjian 1989; Saltzman and Weinmann 1989). Although mapping of essential promoter elements has identified multiple DNA sequences important for template recognition, how the different transcription factors work together with RNA polymerase to select these sequences as sites of initiation is poorly understood. Current results suggest strongly that the DNA sequence specificity of a single protein cannot account for promoter recognition by any of the three cellular RNA polymerases (Yoshinaga et al. 1987; Bell et al. 1989; Murphy et al. 1989; Smale and Baltimore 1989). Instead, it seems that the interactions of multiple proteins, both with the DNA and with one another, are required to generate the observed selectivity of initiation.

The specificity of RNA polymerase I (RNA pol I) transcription is well suited for studies of promoter recognition. Unlike RNA pol II and RNA pol III, which recognize a wide variety of promoters, RNA pol I apparently initiates from only a single type of promoter in the cell, that of the large rRNA gene (Sollner-Webb and Tower 1986). This limited promoter range is reflected in the stringent species specificity of RNA pol I transcription. Studies using either intact cells or in vitro transcription extracts indicate that whereas very closely related species (e.g., mouse and rat) encode functionally interchangeable factors, more distantly related species (e.g., mouse and human) have evolved incompatible promoter recognition properties (Grummt et al. 1982; Onishi et al. 1984). One well-documented exception to this specificity is the finding that the mouse transcription factors are able to initiate transcription from the *Xenopus* rRNA promoter at a site 4 nucleotides upstream from the normal start site (Pape et al. 1990). Comparison of different rRNA promoters indicates that all but the most closely related species share little DNA sequence similarity (Financsek et al. 1982). This rapid evolution of the rRNA promoters is likely to provide a major driving force for compensatory changes in the transcription machinery resulting in the observed transcriptional incompatibility (Dover and Flavell 1984). This changing specificity of the RNA pol I regulatory factors provides an opportunity to investigate the mechanisms governing the selectivity of promoter recognition.

Although RNA pol I transcription exhibits species-specific properties, studies of both the *cis*-acting sequences and *trans*-acting factors required at several different rRNA promoters reveal a number of common

themes. Analysis of vertebrate rRNA promoters indicates that their overall structure is quite similar, with important *cis*-acting elements extending from ~ −200 to just past the start site of transcription (Sollner-Webb and Tower 1986). In humans, this region has been subdivided into two distinct domains: the core promoter element that overlaps the start site of transcription, and an upstream control element (UCE) located between −107 and −187 (Haltiner et al. 1986; Jones et al. 1988). In other species, a clearly defined bipartite promoter structure has not been delineated thus far. However, in all cases studied, deletion of upstream regions (to −50 or more) results in a significantly weakened core promoter, suggesting the presence of additional upstream promoter elements (Sollner-Webb et al. 1983; Miller et al. 1985). Chimeric promoter constructs indicate that the mouse and human core elements are critical for directing species specificity (Learned et al. 1986; Jones et al. 1988; Safrany et al. 1989). Some rRNA genes also contain a series of repetitive sequences upstream of the promoter consisting of multiple duplications of rRNA promoter elements, and for *Xenopus* these sequences have been shown to act as enhancers of RNA pol I transcription (Reeder 1984).

Biochemical studies have also revealed significant similarities between the transcriptional apparatus of different species. In most cases, at least two transcription factors are required in addition to RNA pol I for efficient initiation (Sollner-Webb and Tower 1986). In mammalian species, one of these components is capable of altering the specificity of heterologous extracts (Mishima et al. 1982; Learned et al. 1985). For example, the human factor hSL1, when added to a mouse transcription extract, will reprogram the rodent transcription machinery to recognize both the mouse and the human rRNA promoters. Although hSL1 has no detectable sequence-specific DNA-binding activity on its own, it associates with the human RNA pol I promoter through specific interactions with a second human transcription factor, hUBF (Learned et al. 1986). hUBF is a sequence-specific DNA-binding protein that activates transcription by interacting with the UCE and core elements. The recent cloning of hUBF has identified multiple domains of the protein that exhibit significant homology to the nuclear protein HMG1. Analysis of deletion mutants of the hUBF gene indicates that a subset of these domains is crucial for sequence-specific DNA binding by hUBF (Jantzen et al. 1990). When both hUBF and hSL1 are present, a strong cooperative complex is formed at the promoter that is critical to the transcriptional function of both factors (Bell et al. 1988). Although there are extensive differences between the DNA sequences of rRNA promoters from different species, the DNA-binding specificity of UBF has been conserved (Bell et al. 1989; Dunaway 1989; Pikaard et al. 1989). For example, the *Xenopus* rRNA transcription factor xUBF exhibits identical DNA binding properties to those of hUBF, suggesting that the DNA binding specificity of UBF cannot be responsible for the observed species specificity between human and *Xenopus* (Pikaard et al. 1989). Further comparison of hUBF and xUBF reveals that although DNA binding specificity has been conserved, critical domains for protein–protein interactions have been altered (Bell et al. 1989). Unfortunately, the inability of *Xenopus* and human transcription components to substitute for one another complicates a detailed comparison of the differences between transcription factors from the two species. Instead, we reasoned that analysis of more closely related species might be better suited to study the mechanisms controlling RNA pol I promoter specificity.

Here, we present a comprehensive biochemical analysis of the factors required for mouse and human rRNA transcription. Fractionation of the mouse transcription extract resulted in the identification and purification of a previously unidentified mouse RNA pol I transcription factor. In addition, our studies report the characterization of a separate binding activity associated with mouse SL1. Comparison of these factors with their human equivalents indicates that, despite a number of changes in the affinity of the various individual components for their respective promoters, both the mouse and human rRNA transcription apparati are critically dependent on a cooperative complex consisting of UBF and SL1 for promoter recognition. Moreover, our evidence indicates that only one of the factors involved in this complex is altered to generate the observed specificity of human and mouse rRNA transcription.

Results

Identification of mUBF and mSL1

To study the mechanism of species specificity between mouse and human RNA pol I transcription, we first investigated the components required for transcription and promoter recognition in mouse cells. Using the same fractionation strategy previously employed for the human RNA pol I system, we isolated two components, in addition to RNA pol I, that are required for efficient initiation from the mouse promoter. One factor was a UBF-like DNA-binding activity that we refer to as mouse UBF (mUBF), which was subsequently purified to homogeneity through the use of DNA affinity chromatography (Fig. 1A). The mouse factor, like hUBF, consists of two polypeptides (apparent molecular mass, 94 and 97 kD) that cross-react with antibodies raised against a peptide derived from cloned hUBF (Fig. 1B; Jantzen et al. 1990). In addition, the DNA-binding activities of mUBF and hUBF are indistinguishable on the basis of DNase I footprinting experiments (Fig. 1C,D). The second auxiliary factor has been designated mouse SL1 (mSL1) because, like hSL1, it is essential for initiation and is capable of reprogramming a heterologous extract to recognize the mouse promoter (see below). In addition, mSL1 has similar chromatographic properties to hSL1. The requirements of the various factors for transcription initiation from the mouse rRNA promoter are shown in Figure 2. Similar to the human system, the combination of mSL1 and mouse RNA pol I results in a

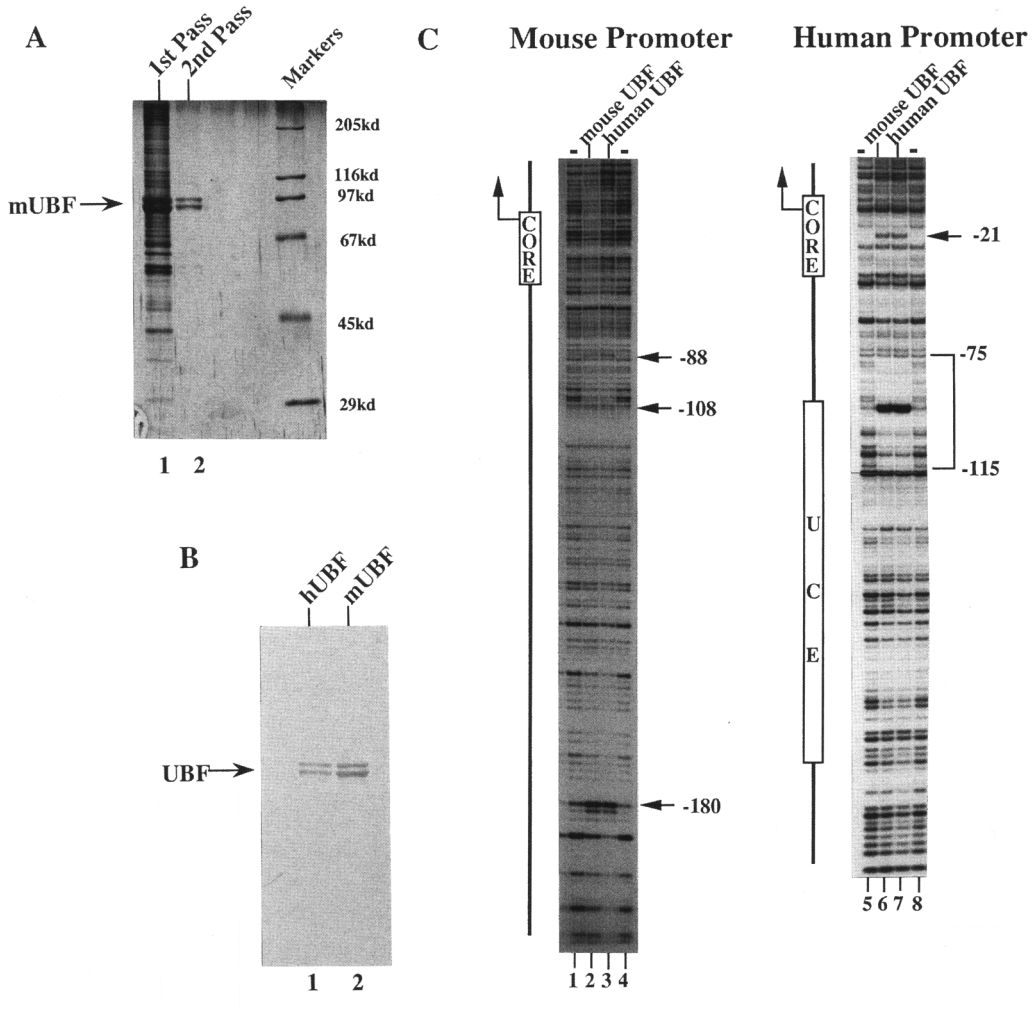

Figure 1. Purification and DNA binding of mUBF. (*A*) Analysis of affinity-purified mUBF by SDS-PAGE followed by silver staining. (Lanes *1* and *2*) The protein composition of the first- and second-pass eluant from the UCE DNA affinity column, respectively. The two mUBF polypeptides are indicated with an arrow, and the sizes of the molecular weight markers are indicated at *right*. All experiments in this paper use second-pass mUBF. (*B*) Western blot analysis with anti-hUBF pep C. Forty nanograms of hUBF (lane *1*) and 60 ng of mUBF (lane *2*) were separated by SDS-PAGE and transferred to nitrocellulose. The protein blot was incubated with anti-hUBF pep C antibody, and antibody–antigen complexes were detected with alkaline phosphatase-conjugated second antibodies. The UBF polypeptides are indicated with an arrow. Controls with preimmune serum show no cross-reactivity (Jantzen et al. 1990). (*C*) DNase I footprinting of purified mUBF and hUBF. Either the noncoding strand of the mouse promoter labeled at −230 and extending to +155 (lanes *1–4*) or the noncoding strand of the human promoter labeled at −208 and extending to +78 (lanes *5–8*) was footprinted with purified mUBF and hUBF. Lanes *1*, *4*, *5*, and *8* had no protein added during DNase I cleavage; lanes *2* and *6* contained 15 ng of affinity-purified mUBF; lanes *3* and *7* contained 15 ng of affinity-purified hUBF. The position of mouse and human promoter elements is indicated to *left* of each footprint. Arrows and brackets indicate enhanced cleavage sites and protected regions, respectively.

low level of transcription, and in the absence of mSL1, no transcription is observed under any conditions. The addition of purified mUBF results in a significant increase (5- to 10-fold) in transcription (Fig. 2, cf. lane 6 with lanes 7 and 8). Neither mSL1 nor mouse RNA pol I preparations exhibit any detectable UBF DNA-binding activity or proteins that cross-react with hUBF-specific antibodies (S.P. Bell, data not shown), suggesting that the low level of transcription in the absence of mUBF is an intrinsic property of these fractions. Interestingly, mUBF alone binds poorly to the mouse template, and even saturating amounts of the factor result in a relatively weak protection of the region between −88 and −108 (Fig. 1D). However, despite its weak affinity for binding independently to the mouse promoter, mUBF is a potent mouse RNA pol I transcription factor.

mSL1 binds DNA in the absence of mUBF

A notable difference between the mouse and human promoter is their relative affinity for UBF. Although both

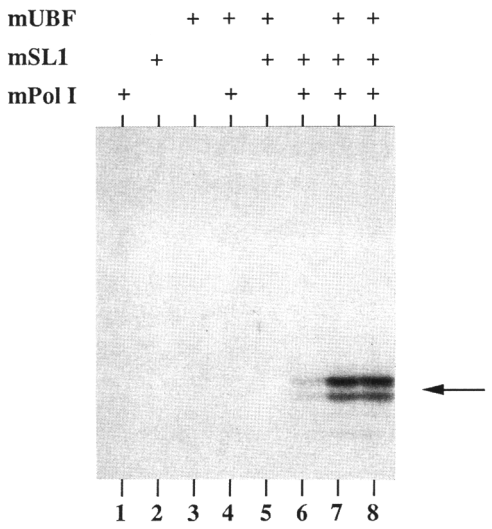

Figure 2. Factors required for mouse RNA pol I transcription. Reconstituted transcription reactions were used to transcribe the mouse wild-type template pSMr −230/+155, and the resulting RNA was analyzed by S1 nuclease protection. Lanes *1*, *4*, *6*, *7*, and *8* contained 0.7 μg of partially purified mouse RNA pol I; lanes *2*, *5*, *6*, *7*, and *8* contained 50 ng of mSL1; lanes *3*, *4*, *5*, *7*, and *8* contained 7.5 ng of purified mUBF. The correct protected fragments of the S1 probe is indicated with an arrow.

mUBF and hUBF bind well to the human promoter, only weak interactions are observed at the mouse promoter. The discrepancy between the weak binding of mUBF and strong transcriptional activation at the mouse promoter suggested that some other component of the mouse transcription apparatus might interact more tightly with the promoter to compensate for the weak interactions of mUBF. Surprisingly, we identified such an activity associated with mSL1. In contrast to hSL1, mSL1 contains a specific DNA-binding activity that recognizes the mouse core promoter element. DNase I footprinting experiments with mSL1 map a protected region between −12 and −52 of the promoter (Fig. 3, lane 4), sequences previously shown to be important for the formation of stable transcription complexes at the mouse promoter (Clos et al. 1986b; Tower et al. 1986; Nagamine et al. 1987). DNA binding experiments have also been performed by use of two point mutations at nucleotides −7 and −16 of the mouse core element (the generous gift of M. Muramatsu). Interestingly, whereas the −7 mutant has no effect on the binding of mSL1 to the promoter, mutation of the promoter at −16 results in a complete loss of mSL1 binding (Fig. 3, cf. lanes 9 and 12). Previous studies indicate that, although both of these mutations reduce transcription, only the −16 mutant has lost the ability to compete with a wild-type promoter for the binding of mouse transcription factors (Nagamine et al. 1987). These results suggest that the binding of mSL1 to the core promoter element plays an important role during mouse rRNA promoter recognition.

mSL1 and mUBF form a cooperative DNA-binding complex

The identification of a DNA-binding activity associated with mSL1 suggested a mechanism by which the weak binding and strong transcriptional activation by mUBF could be reconciled. Just as hUBF mediates the interaction of hSL1 at the human promoter, mSL1 may mediate the interaction of mUBF at the mouse promoter. To test this hypothesis, we performed DNase I footprinting experiments with mUBF and mSL1, either alone or in combination. Consistent with a mechanism involving cooperative binding at the promoter, the combination of mUBF and mSL1 resulted in a dramatically

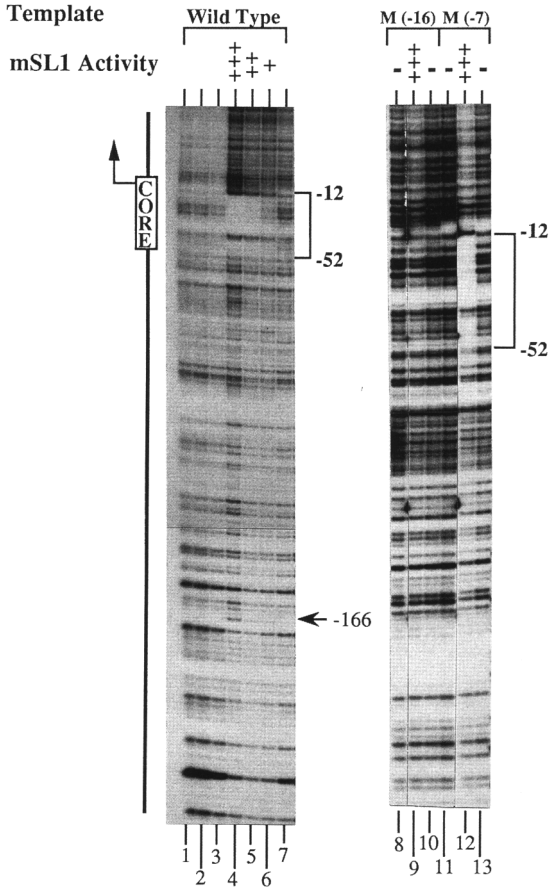

Figure 3. Interaction of mSL1 with the mouse promoter. In lanes *1*–*7* the noncoding strand of the mouse promoter (pSMr −230/+155), labeled at −230 and extending to +155, was used for DNase I footprinting. Each lane contains a 10-μl sample from the mSL1 Mono Q eluant. Lanes *1*–*7* span fractions 4–10 of the elution. The transcriptional activity of each fraction is indicated above each lane by plus signs (+). The noncoding strand labeled at −167 and extending to +155 of the mouse promoter mutants M (−16) (lanes *8*–10) and M (−7) (lanes *11*–*13*) were used for DNase I footprinting. Lanes *8*, *10*, *11*, and *13* contained no protein during DNase I digestion. Lanes *9* and *12* contained 0.25 μg of mSL1. Brackets indicate protected regions; arrows indicate the location of enhanced DNase I cleavage.

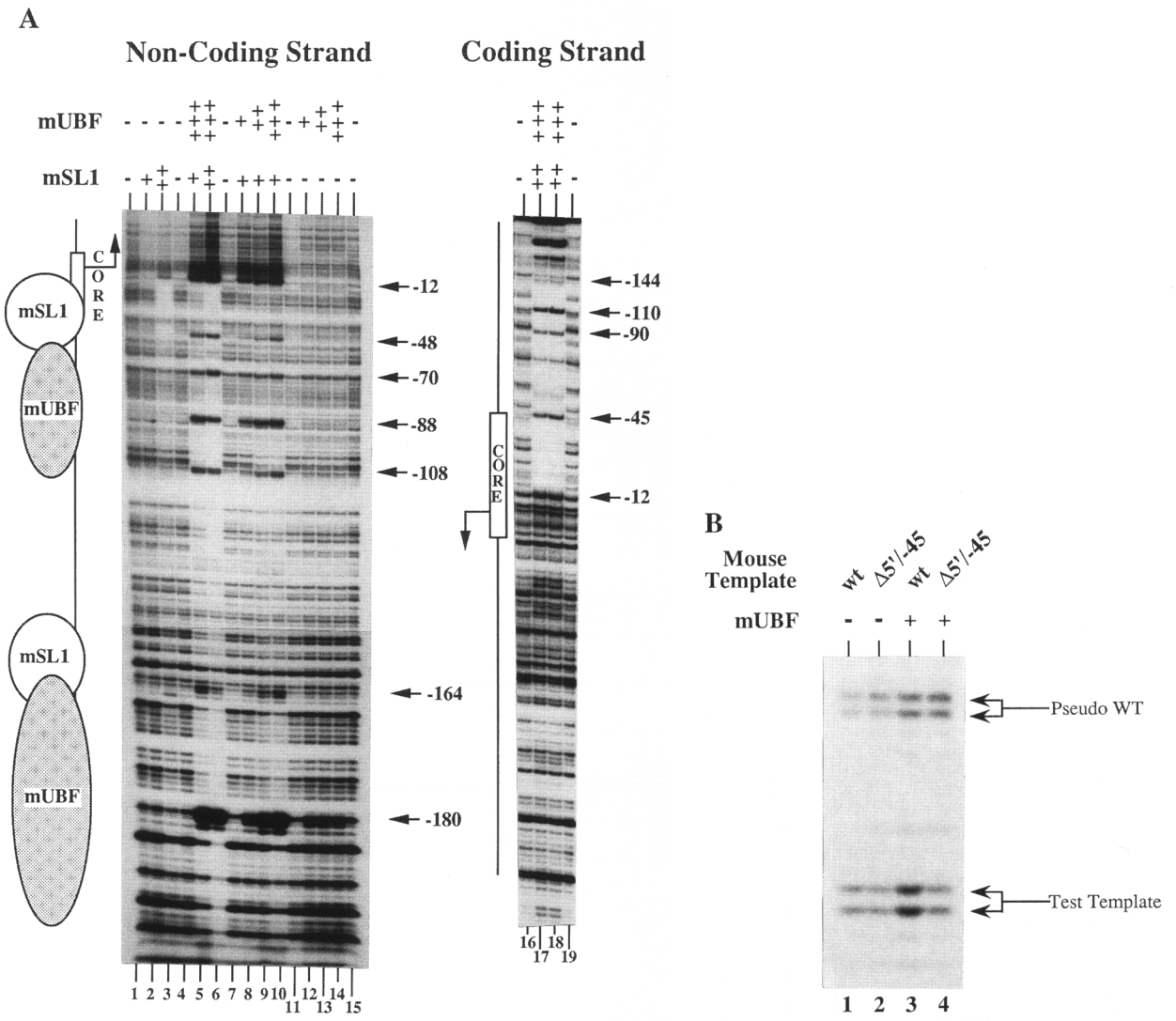

Figure 4. Cooperative DNA binding by mUBF and mSL1. (*A*) The noncoding strand (lanes *1–15*) of the mouse rRNA promoter, labeled as described previously (Fig. 3), or the coding strand (lanes *16–19*) of the mouse promoter, labeled at +155 and extending to −230, were used for DNase I footprinting. Lanes *1*, *4*, *7*, *11*, *15*, *16*, and *19* contained no protein during DNase I digestion. Lanes *5*, *6*, *8*, *9*, *10*, *12*, *13*, *14*, *17*, and *18* contained 2.5 ng (+), 5 ng (++), and 10 ng (+++) of purified mUBF, as indicated above each lane. Lanes *2*, *3*, *5*, *6*, *8*, *9*, *10*, *17*, and *18* contained 125 ng (+) or 250 ng (++) of mSL1, as indicated above each lane. The position of mouse rRNA promoter elements is shown to the *left* of each footprint, as well as the tentative locations of mUBF- and mSL1-binding sites. The positions of enhanced DNase I cleavages are indicated by arrows. (*B*) Dependence of upstream sequences for mUBF activation of transcription. The products of reconstituted transcription reactions were analyzed by an S1 nuclease protection assay. All transcription reactions contained the following: 0.7 μg of partially purified mouse RNA pol I, 50 ng of mSL1, and 30 ng of the template Mo Δ3′/+25. The resulting RNA was analyzed by S1 nuclease protection. Reactions *1* and *3* contained 80 ng of pSMr −230/+155; reactions *2* and *4* contained 80 ng of Mo Δ5′/−45. Reactions *3* and *4* contained 7.5 ng of mUBF. Arrows indicate the correct protected fragments for transcripts arising from the Mo Δ3′/+25 template (pseudo WT), the pSMr −230/+155 (test template), and the Mo Δ5′/−45 template (test template).

enhanced DNase I protection pattern that extends beyond the regions protected by each individual factor (Fig. 4A, cf. lanes 3 and 14 with lane 6). Not only is the normal mSL1 footprint substantially extended toward upstream sequences, but the combination of mUBF and mSL1 also results in a number of new enhanced cleavage sites characteristic of the interaction between UBF and DNA (Fig. 4, lanes 6, 17, and 18). By comparing the binding of the mUBF/mSL1 complex to that of the individual factors, we tentatively identified portions of the extended footprint as either mSL1- or mUBF-binding sites (Fig. 4). Although the cooperative complex binds and protects sequences far upstream of the promoter, there is no change in the cleavage pattern downstream, suggesting that these interactions are sequence specific. Indeed, 5′-deletion mutants that remove upstream sequences prevent binding by the cooperative complex (S.P. Bell, unpubl.). We have also tested the effect of up-

stream deletion mutants on transcriptional activation by mUBF and have found that there is significantly less activation observed for the deletion mutants (Fig. 4B, cf. lanes 2 and 4). These results, taken together with the DNA binding studies, establish that the similarity between mouse and human RNA pol I transcription extends beyond the use of a UBF-binding activity to include cooperativity between UBF and SL1 as a common mechanism of promoter recognition.

hUBF and mUBF are functionally interchangeable

Having characterized the essential components of the mouse transcription apparatus, we then turned to the role of each factor in directing mouse and human promoter specificity. The first issue we addressed was whether hUBF and mUBF are interchangeable with respect to their ability to complex with SL1 and activate transcription. To determine the specificity of these interactions, we asked whether mUBF and hUBF could substitute for each other in the formation of the UBF–SL1 complex (Fig. 5A). Unlike our previous comparison of xUBF and hUBF, both mUBF and hUBF function equally well in the formation of cooperative complexes at either the human promoter with hSL1 or at the mouse promoter with mSL1 (Fig. 5A, cf. lanes 6 and 7 with lanes 15 and 16). The ability of hUBF and mUBF to substitute for each other in the formation of these complexes predicts that the two proteins may also be capable of activating transcription equally well from both promoters. Indeed, we find that adding either mUBF or hUBF to a transcription reaction containing hSL1, human RNA pol I, and a human template leads to an equivalent level of activation by both factors (Fig. 5B). Similar results are obtained at the mouse promoter (Fig. 5C). Thus, mUBF and hUBF have conserved not only their DNA binding properties but also the domains required for transcriptional activation and productive interactions with SL1. Furthermore, despite the apparent role of UBF in promoter recognition by RNA pol I, the interchangeability of mUBF and hUBF argues that they are not responsible for human and mouse species specificity.

Species-specific activities of SL1

The functional similarity between mUBF and hUBF suggests that SL1 is responsible for the promoter selectivity of rRNA transcription. Such a hypothesis implies both that SL1 interacts with the DNA directly and that the interaction of SL1 with the promoter should be species specific. To test this, we performed DNA binding experiments with hSL1 at the mouse promoter and mSL1 at the human promoter. The results indicate that neither mSL1 nor hSL1 interacts specifically with the heterologous promoter (cf. Fig. 6, lanes 4 and 12 with Fig. 5A, lanes 4 and 14). This finding also demonstrates that the different binding properties of mSL1 and hSL1 are not due to the presence of a low-affinity binding site at the human promoter versus a high-affinity site at the mouse promoter.

We then tested whether hSL1 and mSL1 could substitute for each other in the formation of the UBF–SL1 complex at a heterologous promoter. Such an experiment was particularly important in the case of hSL1, as it does not bind independently to either the mouse or human promoter. We find that the binding of such heterologous complexes results in DNase I protection patterns that are only a subset of those observed with the homologous SL1 (cf. Fig. 6, lanes 6, 7, 14, and 15 with Fig. 5A, lanes 6, 7, 15, and 16). However, the protection patterns differ from those seen with UBF alone. This difference is most striking at the mouse promoter, where the addition of hSL1 results in the formation of at least two new protected regions. Interestingly, these regions are centered around enhanced cleavages seen with UBF alone (at −88, −108, and −180), suggesting that the new protected regions are due to the enhancement of UBF binding. This finding, as well as the weak enhancement of the normal UBF footprint at the human promoter by mSL1 (Fig. 6; cf. lanes 14 and 15 with lanes 10 and 11), suggests that some subset of the normal UBF–SL1 interaction can occur even in the absence of SL1-binding sites. Most importantly, the species-specific DNA recognition properties of SL1 suggest a direct role for this factor in determining mouse and human rRNA promoter specificity.

A complementary experiment to address the role of SL1 and other transcription factors in species specificity is to perform transcription reactions with all the different combinations of mouse and human factors and determine what combinations are active and at which promoter. When all eight permutations of the factors (i.e., SL1, UBF, and RNA pol I) are used to transcribe the mouse promoter (Fig. 7A), only those reactions that contain mSL1 result in specific initiation from the mouse promoter, regardless of the origin of the UBF and RNA pol I. Similarly, when the same transcription reactions are performed with the human template (Fig. 7B), only those reactions containing hSL1 recognize and initiate RNA synthesis from the human promoter. Some quantitative differences are observed in the efficiency of transcription catalyzed by the various combinations of factors. However, these differences appear to be dependent on the the origin of the RNA pol I, with the mouse enzyme exhibiting consistently weaker transcription than its human counterpart. Taken together, these findings indicate that the source of UBF and RNA pol I do not contribute significantly to species specificity and, instead, strongly support the conclusion that the origin of SL1 activity is responsible for determining the specificity of rRNA transcription.

Discussion

A thorough understanding of promoter recognition in eukaryotic cells requires both the identification of the *trans*-activators involved and a detailed mechanistic insight of how these factors interact with the DNA, each

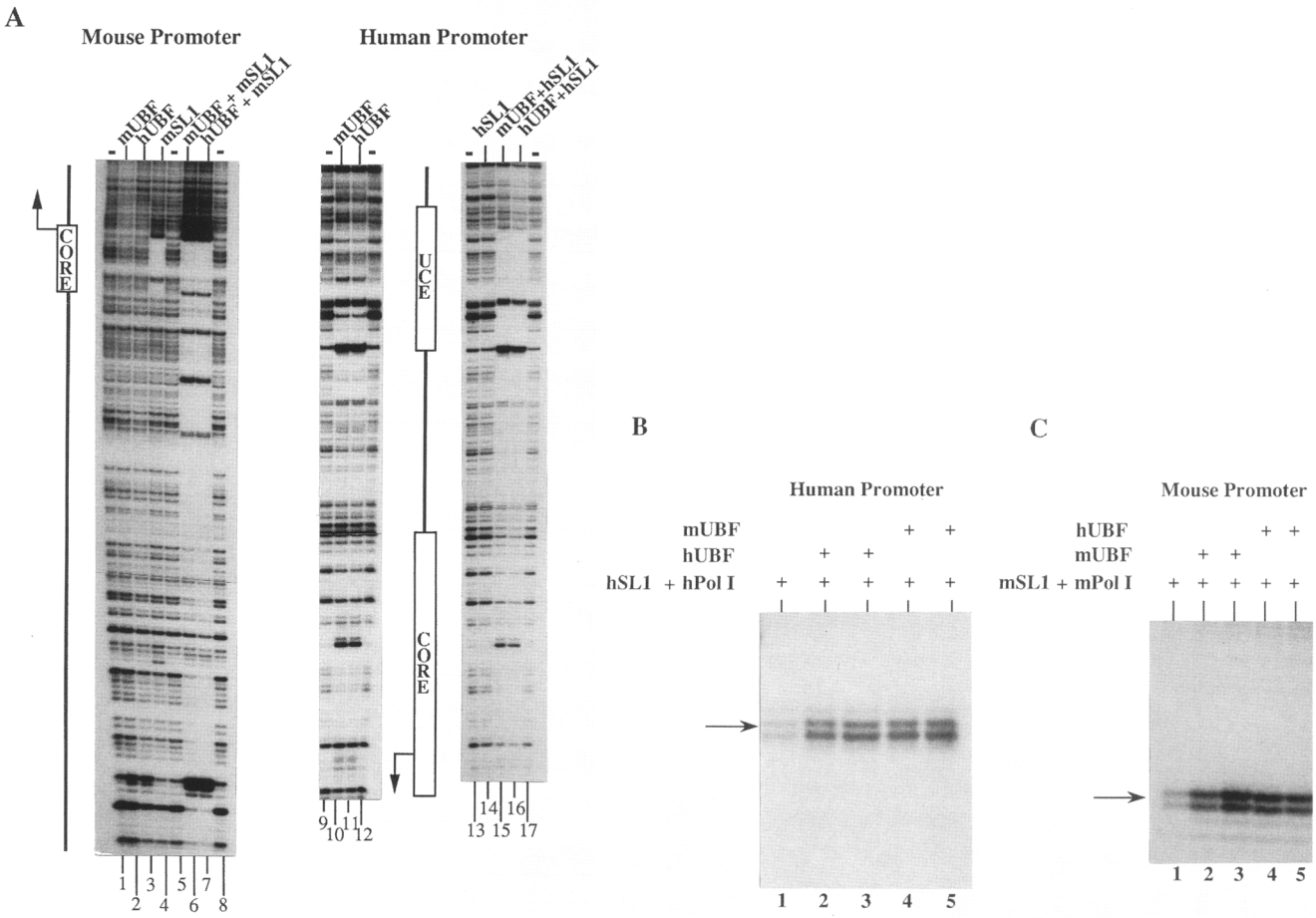

Figure 5. mUBF and hUBF are interchangeable. (*A*) hUBF and mUBF both bind cooperatively with hSL1 and mSL1. Either the noncoding strand of mouse rRNA promoter (lanes *1–8*), labeled as described previously (Fig. 3), or the coding strand of the human promoter (lanes *9–17*), labeled at +24 and extending to −500, was used for DNase I footprinting. Lanes *1*, *5*, *8*, *9*, *12*, *13*, and *17* contained no protein during DNase I digestion. Lanes *2*, *6*, *10*, and *15* contained 10 ng of purified mUBF. Lanes *3*, *7*, *11*, and *16* contained 10 ng of purified hUBF. Lanes *4*, *6*, and *7* contained 250 ng of mSL1. Lanes *14–16* contained 1 μg of hSL1 and 1 μg of dAT competitor DNA. All other lanes contained 0.1 μg of dAT competitor DNA. Diagrams to *left* of the mouse footprint and between the two human footprints show the location of promoter elements. (*B*) hUBF and mUBF both activate transcription from the human rRNA promoter. Reconstituted transcription reactions were performed, and the resulting RNA was analyzed by S1 nuclease protection. All reactions contained 10 ng of hSL1 and 0.8 μg of partially purified human RNA pol I and 100 ng of the template prHu3. Reactions *2* and *3* contained 7.5 ng of purified hUBF; reactions *4* and *5* contained 7.5 ng of purified mUBF. (*C*) hUBF and mUBF both activate transcription from the mouse rRNA promoter. Reconstituted transcription reactions were performed, and the resulting RNA was analyzed by S1 nuclease protection. All reactions contained 50 ng of mSL1, 0.7 μg of partially purified mouse RNA pol I, and 100 ng of the mouse template (pSMr −230/+155). Reactions 2 and 3 contained 7.5 ng of purified hUBF; reactions 4 and 5 contained 7.5 ng of purified mUBF. Arrows indicate the correct protected fragments for transcripts arising from each promoter.

other, and RNA polymerase. In this study, we have taken advantage of the rapidly evolving specificity of RNA pol I promoter recognition to investigate the differences in the mouse and human transcription machinery that result in their distinct promoter specificities. Our findings indicate that both mouse and human cells mediate rRNA promoter recognition through the concerted action of two factors, UBF and SL1. Interestingly, we find that only SL1 has altered its DNA recognition properties between mouse and human cells, whereas UBF has remained apparently unchanged. Furthermore, the protein–protein interactions between factors appear to be strongly conserved. Thus, our studies suggest strongly that the divergent recognition properties of SL1 are responsible for the species specificity exhibited by mouse and human rRNA transcription. These findings therefore demonstrate the ability of multiprotein complexes to direct promoter recognition and illustrate how specific interactions between proteins can generate functional diversity during eukaryotic transcription (see below).

Role of the UBF–SL1 complex in promoter recognition

Our current model for species specificity of mouse and human rRNA transcription is illustrated in Figure 8A.

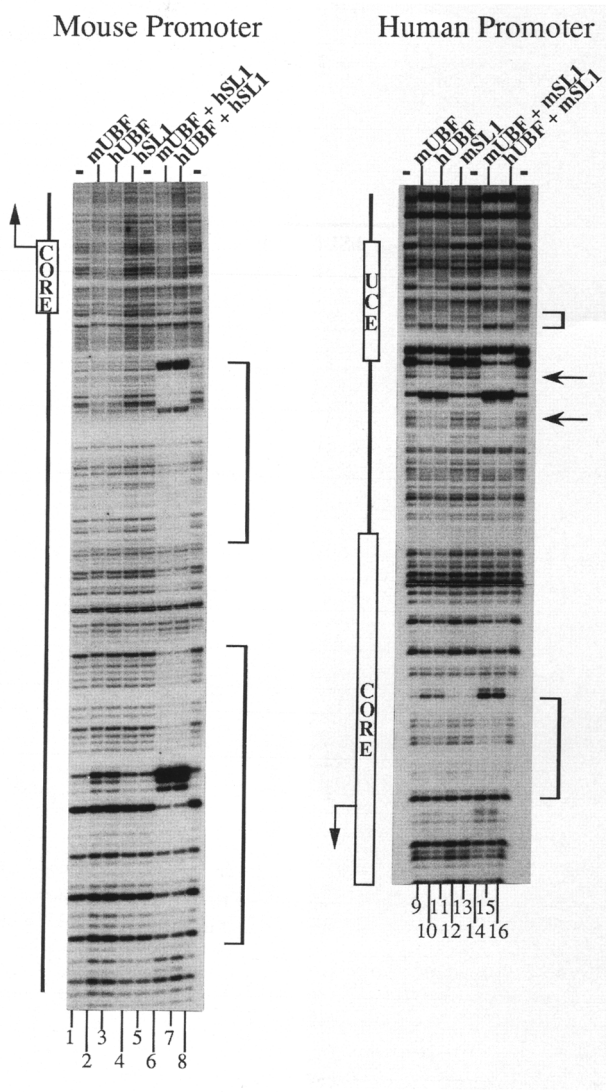

Figure 6. mSL1 and hSL1 cannot substitute for each other in DNA-binding assays. The noncoding strand of the mouse rRNA promoter (lanes *1–8*) or the coding strand of the human promoter (lanes *9–16*), labeled as described previously (Fig. 5), were used for DNase I footprinting. Lanes *1*, *5*, *8*, *9*, *13*, and *16* contained no protein during DNase I digestion. Lanes *2*, *6*, *10*, and *14* contained 10 ng of purified mUBF. Lanes *3*, *7*, *11*, and *15* contained 10 ng of purified hUBF. Lanes *4*, *6*, and *7* contained 1 μg of hSL1. Lanes *12*, *14*, and *15* contained 250 ng of mSL1. Diagrams to *left* of each footprint indicate the location of mouse and human promoter elements. Brackets and arrows to *right* of each footprint indicate the regions of enhanced protection seen in the presence of SL1.

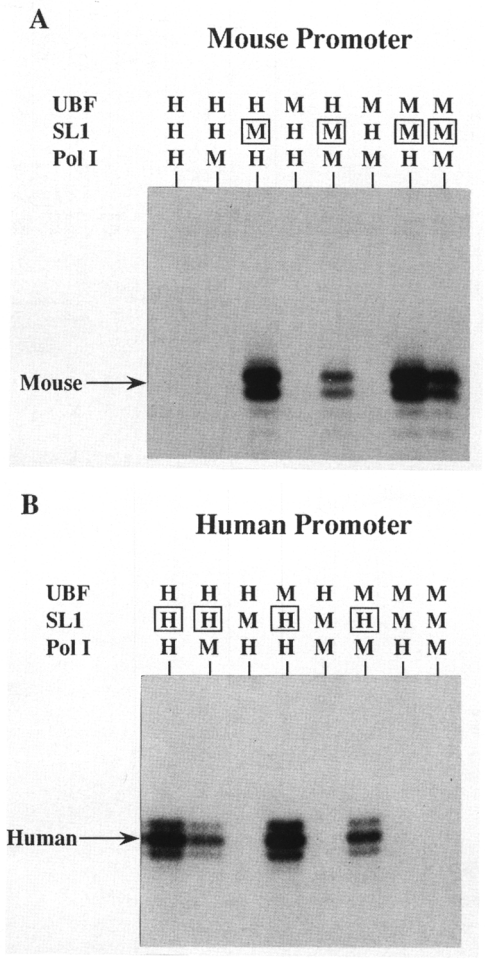

Figure 7. SL1 directs the promoter specificity of reconstituted transcription reactions. Each reaction contained the mouse (M) or human (H) transcription components, as indicated above each lane in the following amounts: RNA pol I, either 0.8 μg of partially purified human RNA pol I (H) or 0.7 μg of partially purified mouse RNA pol I (M); SL1, either 10 ng of hSL1 (H) or 50 ng of mSL1 (M); UBF, 10 ng of either hUBF (H) or mUBF (M). (*A*) The results of S1 nuclease analysis of RNA produced in reactions containing 100 ng of mouse template (pSMr −230/+155). (*B*) The results of S1 nuclease analysis of RNA produced in reactions containing 100 ng of human template (prHu3). Arrows indicate the correct protected fragments for transcripts arising from each promoter. Boxed SL1 letters (M or H) indicate reactions directing specific initiation.

At a homologous promoter, UBF and SL1 (from the same species) form a productive complex that is recognized by RNA pol I, resulting in initiation of transcription. In contrast, we propose that at a heterologous promoter, despite the ability of UBF and SL1 to form significant protein–protein interactions, the subsequent interaction of SL1 with the DNA is disrupted. The inability of SL1 to interact correctly with the promoter leads to the formation of a nonproductive complex that is unable to mediate transcription. A previous comparison of human and *Xenopus* UBF identified significant differences in the ability of the two proteins to activate transcription and to interact with hSL1, strongly suggesting that these changes are involved in the species specificity of human and *Xenopus* rRNA transcription (Bell et al. 1989). In contrast, the findings presented here suggest that the inability of mammalian UBF and SL1 to function at a heterologous promoter is not controlled by changes in the protein–protein interactions between the factors. Instead, the specificity appears to be due to the inability of SL1 to interact correctly with species-specific elements

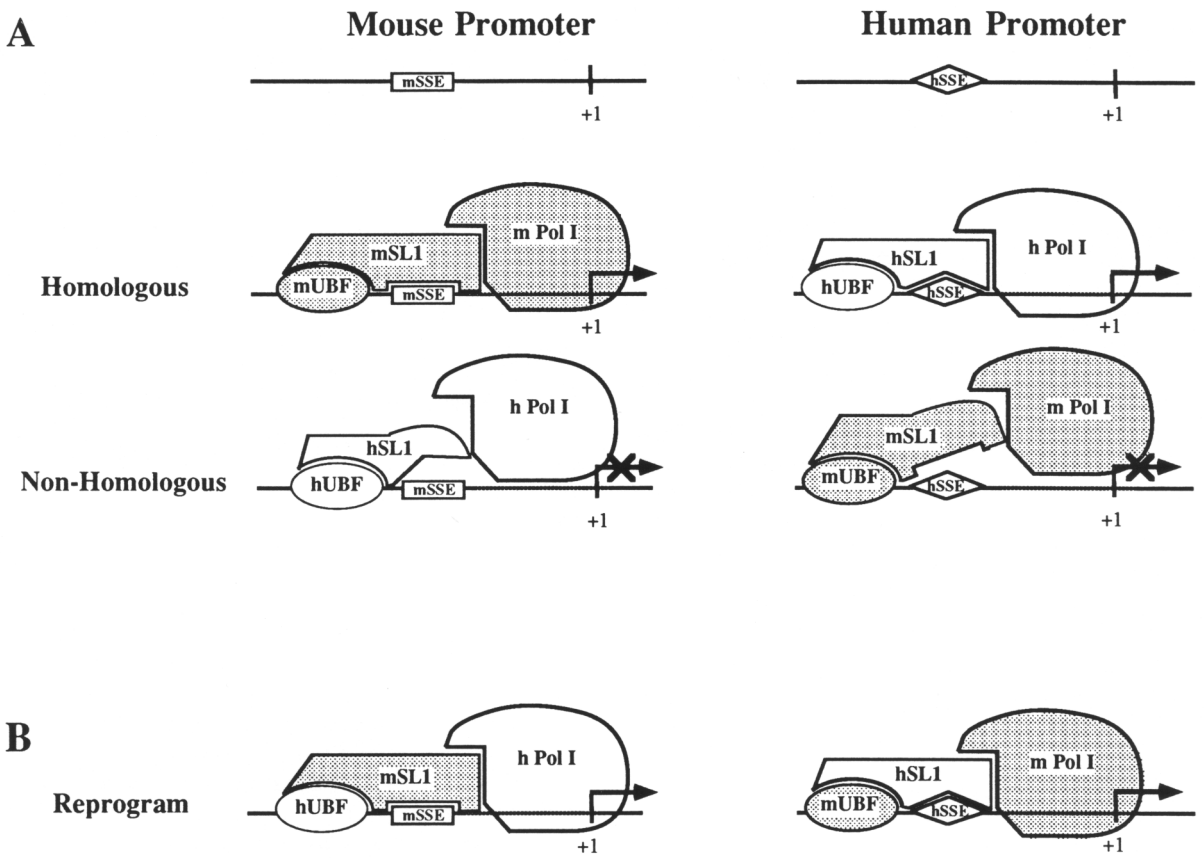

Figure 8. A model for mouse and human RNA pol I promoter recognition. (*A*) Species specificity of promoter recognition. We hypothesize that when all of the factors from a particular species are present at their cognate promoter (*top row*), a normal UBF–SL1 complex is formed, with SL1 recognizing the SSE (Safrany et al. 1989), and this complex is capable of directing initiation by RNA pol I. In contrast, if factors from one species are present at the heterologous promoter (*bottom row*), a nonproductive UBF–SL1 complex is formed due to the inability of the SL1 to recognize the heterologous SSE, and this complex is unable to direct initiation by RNA pol I. (*B*) Reprogramming of heterologous extracts by SL1. We predict that reprogramming of heterologous extracts by hSL1 and mSL1 involves the formation of heteromeric UBF–SL1 complexes with the endogenous UBF present in the extract and the exogenously added SL1. Such a complex has the ability to recognize the SSE compatible with the added SL1 and redirect the endogenous RNA pol I to initiate from this promoter. Mouse transcription factors are shaded; human factors are not. (hSSE and mSSE) Human and mouse SSEs, respectively. One determinant of an SSE may be the location or orientation of the UBF-binding sites. For the purpose of simplicity, RNA polymerase is shown interacting with SL1 only; however, we do not rule out direct interactions between UBF and the polymerase. An X over the start site arrow is indicative of loss of transcription.

(SSEs) of the different promoters. Several lines of evidence support this conclusion. First, we demonstrate that both hSL1 and mSL1 exhibit species-specific DNA recognition properties in the presence or absence of UBF. Moreover, the sequences that mSL1 protects from DNase I cleavage are very similar to the sequences defined previously as SSEs by use of human and mouse promoter chimeras (Safrany et al. 1989). In contrast, UBF from both species shows interchangeable DNA binding and transcriptional activation properties. Second, the origin of SL1, and not of UBF or RNA pol I, determines the transcriptional specificity of reconstituted transcription reactions. Finally, both the interchangeability of UBF at the mouse and human promoters and the ability of SL1 to enhance the binding of UBF at heterologous promoters suggest that the domains involved in protein–protein interactions have been conserved.

The ability of mSL1 and hSL1 to interact with the transcription machinery of both species undoubtedly plays a central role in the process of transcriptional reprogramming (Fig. 8B; Mishima et al. 1982; Learned et al. 1985). We propose that, for example, the addition of hSL1 to a mouse extract results in the formation of a heteromeric mUBF–hSL1 complex in the presence of the human promoter. This complex is then competent to redirect mouse RNA pol I to initiate transcription from the human promoter. The finding that hSL1, mUBF, and mouse RNA pol I together transcribe the human promoter indicates that such a heteromeric complex can direct initiation by the mouse polymerase.

The DNA binding properties of mSL1 are easily recognized. However, in the human system, species-specific binding can only be detected for the hUBF–hSL1 complex. Although our current studies do not demonstrate a specific interaction of hSL1 alone with DNA, two lines of evidence argue that such interactions are important

for hSL1 function. First, the overall similarity of mouse and human RNA pol I transcription and the clear role of a DNA-binding activity in mSL1 activity imply that hSL1 is also likely to interact with DNA in a specific manner. Second, previous analysis indicates that mutations not affecting UBF binding at the human promoter nevertheless cause dramatic inhibition of the subsequent formation of the UBF–SL1 complex, suggesting that a second DNA-binding activity is involved in the formation of the complex (Learned et al. 1986). We hypothesize that like prokaryotic σ-factors (Helman and Chamberlin 1988), hSL1 interacts with the DNA in a specific manner but does so only in the presence of a secondary factor, in this case, hUBF.

Previous studies of mammalian rRNA transcription have identified several activities with properties reminiscent of UBF and SL1. DNase I footprinting studies of TF-ID, an activity identified in both mouse and human cells, shows very similar footprinting patterns to those observed with reactions containing a combination of UBF and SL1 at their cognate rRNA promoters (Safrany et al. 1989). In addition, like SL1, TF-ID can reprogram heterologous transcription extracts (Mishima et al. 1982). Two other activities, factor D and TIF-IB, both identified in mouse cells, appear to be related to mSL1 activity. Mapping of the sequences required for the formation of stable transcription complexes by factor D or protected from exonuclease III digestion by TIF-IB, results in the identification of sequences that are protected by mSL1 during DNase I protection assays (Clos et al. 1986a; Tower et al. 1986). In addition, mutants that prevent binding of mSL1 also prevent binding of TIF-IB in vitro and stable transcription complex formation by TF-ID (Fig. 3 and S.P. Bell, unpubl.; Clos et al. 1986a,b; Nagamine et al. 1987). Thus, it is likely that mSL1 is an important component for TF-ID, TIF-IB, and factor D function. Although footprinting studies strongly suggest that mUBF is also a component of TF-ID, the role of UBF in TIF-IB and factor D function is not clear from current studies. The exact identity of these various fractions awaits the molecular identification of the polypeptide(s) that controls the function of mSL1 and the other factors.

Mechanisms for the generation of differential specificity

Recent studies of RNA pol II transcription have identified groups of regulators that recognize identical DNA binding sites but apparently do not perform the same function in the cell. Such families of factors include the c-*jun* proto-oncogene product and its homologs, various *Drosophila* homeo domain proteins such as the products of *zen*, *eve*, and *en*, and the POU domain proteins Oct-1, Oct-2, and Pit-1 (Curran and Franza 1988; Herr et al. 1988; Hoey and Levine 1988). How does the cell regulate and control the function of these factors in light of their nearly identical template recognition properties? Studies of UBF and SL1 serve to illustrate two mechanisms through which functional diversity can be generated by proteins with identical recognition properties. Comparison of hUBF and xUBF strongly suggests that the specificity of these factors is due to their different interfaces for protein–protein interaction. Similarly, it appears that differences in the protein–protein interaction domains of Oct-1 and Oct-2 direct VP16 to activate transcription only in conjunction with Oct-1 (Stern et al. 1989). Other RNA pol II transcriptional regulators with identical binding properties, such as homeo domain-containing proteins, may also have distinct interfaces for protein–protein interactions, thereby allowing each factor to select out different partners and to perform its appropriate functions (Levine and Hoey 1988).

A second mechanism to generate alternate specificities of transcription by common DNA-binding proteins is illustrated by the UBF and SL1 interactions at the mouse and human promoters. Despite the apparent interchangeability of mouse and human UBF, they can be involved in complexes at different templates, depending on the origin of the associated SL1. Thus, UBF is directed to function at the mouse promoter in the presence of mSL1, and UBF activates the transcription of the human promoter in the presence of hSL1. Likewise, RNA pol II regulatory proteins may also be capable of interacting with multiple secondary factors leading to recognition of different promoters and potentially different transcriptional outcomes. In contrast to the previous model, this mechanism has the potential to allow a single protein to perform a number of different functions by associating with different auxiliary factors. The role of the yeast MCM1 protein in cell-type determination represents another example of a single protein generating specificity through multiple factor–factor interactions. Depending on the yeast cell type, MCM1 may interact with either α2 or **a**1, resulting in complexes that recognize different binding sites and either repress or activate transcription, respectively (Herskowitz 1989).

RNA pol II promoters generally require multiple *cis*-acting elements capable of directing the initiation of transcription, including the TATA box (Breathnach and Chambon 1981; Dynan and Tjian 1985) and the more recently identified initiator (Smale and Baltimore 1989). Biochemical evidence suggests that the lack of a TATA box at a promoter does not circumvent the requirement for TFIID, the TATA-binding activity (Carcamo et al. 1989; F. Pugh, pers. comm.). This finding is reminiscent of the observation that UBF is still needed to activate transcription from the mouse promoter, even though it binds the template very weakly. It is possible that like UBF, TFIID may be brought into the initiation complex, at least partially, by protein–protein interactions with other factors involved in promoter recognition (Buratowski et al. 1989; Conaway and Conaway 1989). The use of such a mechanism may, in part, explain the diverse recognition properties of RNA pol II transcription. We find that, in the case of RNA pol I, strong binding by the UBF–SL1 complex requires a high-affinity binding site for one but not both of the interacting factors. Likewise, the RNA pol II transcription machinery may require only a subset of the potential protein–DNA interactions available to select a promoter sequence, thereby

allowing significantly greater flexibility in the promoters recognized. We anticipate that the study of the UBF–SL1 complex and its role in promoter recognition will provide a useful model for elucidating the mechanisms of multiprotein complexes in DNA recognition events involved in both transcription and other nuclear processes.

Materials and methods

Protein purification

Human transcription factors were purified as described previously (hUBF and RNA pol I, Bell et al. 1988, 1989; hSL1, Learned et al. 1986). Mouse transcription factors were purified as follows. Nuclear extract (Dignam et al. 1983; Briggs et al. 1986) was prepared from mouse lymphocyte-derived L1210 cells, except that KCl was substituted for NaCl. Extract from 36 liters of cells [typical total protein was 1.4 g, as measured by Bradford assay (Bio-Rad) with gamma globulin standard] was loaded onto a 100-ml heparin–agarose column equilibrated with TM [50 mM Tris-HCl (pH 7.9), 12.5 mM $MgCl_2$, 20% glycerol, 0.5 mM EDTA, 1 mM DTT, and 0.1 M KCl]/0.1 M KCl and eluted with an 800-ml salt gradient between 0.2 and 0.65 M KCl. The peak of RNA pol I and mUBF activity eluted at 0.27 M KCl and 0.34 M KCl, respectively. Further purification of these activities was essentially as described for their human counterparts (Bell et al. 1988, 1989). The peak of mSL1 activity (eluted at 0.48 M KCl) was pooled and dialyzed against TM^{10}/0.09 M KCl (TM^{10} buffer is identical to TM buffer except that it contains 10% glycerol). This material was loaded onto a 1-ml Mono Q (Pharmacia) column equilibrated with TM^{10}/0.09 M KCl and eluted with a 22-ml salt gradient between 0.09 and 0.45 M KCl. Peak mSL1 activity was eluted at 0.14 M KCl. Both hSL1 and mSL1 and RNA pol I fractions were found to have no detectable UBF-binding activity. In addition, these fractions contained no proteins that cross-reacted with anti-UBF antibodies.

In vitro transcription

In vitro transcription reactions were performed as described previously (Learned et al. 1986; Bell et al. 1988). All templates were double-stranded supercoiled plasmids. All human transcription reactions were directed by the plasmid prHu3 (Learned et al. 1985). The wild-type mouse template (pSMr −230/+155) was a subclone of the −230 to +155 fragment (*Eco*RI–*Sma*I) of the plasmid Δ5′/−230 (Henderson and Sollner-Webb 1986) into Bluescript SK^+ (Stratagene). Further deletion mutants of this template were generated by using exonuclease III digestion (Henikoff 1987). The mouse pseudo-wild-type template was a 3′-deletion mutant (deleted to +25) and exhibited transcriptional activity equivalent to the wild-type promoter. The resulting RNA was analyzed by S1 nuclease analysis with 5′-end-labeled single-stranded DNA oligomers. The mouse wild-type probe was homologous to the region from −20 to +30 of the mouse promoter, and the human probe was homologous to the region between −20 to +40 of the human promoter. The mouse pseudo-wild-type probe was homologous to a region between −20 and +40 of the pseudo-wild-type template (including 15 nucleotides of the Bluescript polylinker, allowing the distinction from wild type).

Footprinting analysis

Footprinting was performed as described previously. All reactions contained 0.1 μg of dAT competitor (Sigma), unless otherwise noted. Typically, 3–5 fmoles of template was used per reaction. Human templates used were pSBr208 labeled at −208 (noncoding strand) and pSBr24 (Bell et al. 1989) labeled at +24 (coding strand). The mouse wild-type template (pSMr −230/+155) was labeled by use of sites in the Bluescript polylinker at either −230 (noncoding strand) or +155 (coding strand). The mouse point mutations −7 and −16 (Safrany et al. 1989) were the generous gifts of M. Muramatsu and were labeled at the *Hin*dIII site at −167.

Protein analysis

All protein samples were TCA-precipitated, washed with acetone, and resuspended in sample buffer before SDS–PAGE (Laemmli 1970). Proteins were visualized by silver staining or Western blotting (Jantzen et al. 1990).

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Assembly of alternative multiprotein complexes directs rRNA promoter selectivity.

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