The *Xenopus* RNA polymerase I transcription factor, UBF, has a role in transcriptional enhancement distinct from that at the promoter

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Repeated sequence elements found upstream of the ribosomal gene promoter in *Xenopus* function as RNA polymerase I-specific transcriptional enhancers. Here we describe an *in vitro* system in which these enhancers function in many respects as *in vivo*. The principal requirement for enhancer function *in vitro* is the presence of a high concentration of upstream binding factor (UBF). This system is utilized to demonstrate that enhancers function by increasing the probability of a stable transcription complex forming on the adjacent promoter. Species differences in UBF are utilized to demonstrate that enhancers do not act by recruiting UBF to the promoter, rather UBF performs its own distinct role at the enhancers. UBF function in enhancement differs from that at the promoter, as it is flexible with respect to both the species of UBF and the enhancer element employed. Additionally, we identify a potential role for the mammalian UBF splice variant, UBF2, in enhancer function. We demonstrate that the TATA box binding protein (TBP)-containing component of *Xenopus* RNA polymerase I transcription, Rib1, can interact with an enhancer-UBF complex. This suggests a model in which enhancers act by recruiting Rib1 to the promoter.

Keywords: enhancers/RNA polymerase I/UBF/*Xenopus*

Introduction

Transcription of the repeated genes that encode 18S and 28S rRNAs by RNA polymerase I (RNA pol I) requires a combination of *cis*-acting regulatory sequences and *trans*-acting protein factors that interact with these sequences (Reeder, 1992). Regulatory sequences include promoter and enhancer elements. *Xenopus* enhancer elements comprise blocks of interspersed 60 and 81 bp repeats that have sequence homology to the gene promoter (Busby and Reeder, 1983; Moss, 1983; Reeder *et al.*, 1983; Labhart and Reeder, 1984, 1985). *Xenopus* oocyte injection experiments demonstrate that blocks of these elements confer a competitive advantage on a linked promoter. Repetitive sequence elements are also found in the intergenic spacer, immediately upstream of the gene promoter, in rodent species. In the mouse, these repeated elements are 140 bp in length and have been demonstrated to have enhancer activity in both mouse and *Xenopus* systems (Kuhn *et al.*, 1990; Pikaard *et al.*, 1990). Unlike

Xenopus, mouse enhancer elements have no sequence similarity with the gene promoter. A sequence element with enhancer function has also been identified in the yeast intergenic spacer (Schultz *et al.*, 1993).

RNA pol I transcription minimally requires the *trans*-acting factors upstream binding factor (UBF) and a second factor termed SL1 in humans (Bell *et al.*, 1988), TIF 1B (Schnapp *et al.*, 1990) or Factor D (Mishima *et al.*, 1982; Tower *et al.*, 1986) in mouse and Rib1 in *Xenopus* (McStay *et al.*, 1991a). For reasons of clarity, we will collectively refer to this second factor in mammals as SL1. Human SL1 is comprised of TATA box binding protein (TBP) and TBP-associated factors (TAF_{II}s) of 48, 63 and 110 kDa (Comai *et al.*, 1992, 1994; Zomerdijs *et al.*, 1994). Likewise, mouse SL1 is comprised of TBP and TAF_{II}s of 48, 68 and 95 kDa (Eberhard *et al.*, 1993). Recently, we have demonstrated that Rib1 is comprised of TBP and TAF_{II}s (Bodeker *et al.*, 1996). However, the size and number of these TAF_{II}s has not yet been determined.

UBF, which binds to DNA sequences within the promoter (Bell *et al.*, 1988; Pikaard *et al.*, 1989), has been termed an 'architectural' transcription factor because of its remarkable propensity to bend and loop DNA (Reeder *et al.*, 1995). Electron spectroscopic imaging has demonstrated that a single dimer of *Xenopus* UBF (xUBF) can organize ~180 bp of DNA into a loop of almost 360° (Bazett-Jones *et al.*, 1994; Stefanovsky *et al.*, 1996). A ligase-mediated probe circularization assay was used to show the ability of xUBF to loop short DNA fragments (Putnam *et al.*, 1994). This architectural ability is conferred by the presence of multiple HMG box DNA binding motifs in UBF (Jantzen *et al.*, 1990; McStay *et al.*, 1991b; Hu *et al.*, 1994). HMG box proteins have also been characterized as key architectural elements in RNA polymerase II systems (for review, see Grosschedl *et al.*, 1994).

SL1 on its own binds very poorly to DNA, but in the presence of human UBF (hUBF) it binds tightly and specifically to DNA sequences within the human promoter (Bell *et al.*, 1988). Similarly, xUBF and Rib1 can combine to form a stable transcription complex on the *Xenopus* promoter (McStay *et al.*, 1991a). The interaction of Rib1 and SL1 with their respective promoters requires precise architecture of UBF. This is demonstrated by the observation that a UBF molecule with an inappropriate number of HMG boxes (generated by the deletion or insertion of additional HMG boxes) is non-functional in transcription (Bell *et al.*, 1989; McStay *et al.*, 1991b; Jantzen *et al.*, 1992; Cairns and McStay, 1995).

In addition to binding to DNA sequences within the promoter, UBF can give rise to a DNase I footprint over *Xenopus* (Pikaard *et al.*, 1989) and mouse (Pikaard *et al.*, 1990) enhancer sequences. Addition of a large molar excess of enhancer elements to transcription reactions can inhibit transcription initiation (Pikaard *et al.*, 1989; Kuhn

et al., 1990). This competitive effect can be relieved partially by the addition of excess UBF (Pikaard *et al.*, 1989). These observations have been interpreted as evidence for the involvement of UBF in enhancer function (Pikaard *et al.*, 1989). To date, there has been no direct evidence for the involvement of UBF in enhancer action. Here we describe an *in vitro* system in which enhancers function in many respects as *in vivo*. This system has allowed us for the first time to demonstrate directly the involvement of UBF in *Xenopus* enhancement and to also show that enhancers act by increasing the probability of stable transcription complex formation on a linked promoter.

One can imagine two classes of model of enhancer action with respect to UBF. Enhancers may act by recruiting UBF to the promoter. Alternatively, UBF may perform its own distinct role at the enhancer that does not involve translocation to the promoter. Here we have exploited the observed species specificity of UBF in promoter function to distinguish between these two models and demonstrate that UBF does indeed have a role in enhancement distinct from that at the promoter. We demonstrate that Rib1 can interact with an enhancer-UBF complex. This suggests a model of enhancer action in which an enhancer-UBF complex recruits Rib1 to the promoter. Additionally, we show that mammalian UBF2, previously shown not to function at mammalian promoters (Jantzen *et al.*, 1992; Kuhn *et al.*, 1994), may have a role in enhancer function.

Results

High concentrations of xUBF facilitate enhancer function *in vitro*

S100 extracts prepared from *Xenopus* culture cells support accurate and efficient transcription initiation by RNA pol I (McStay and Reeder, 1986). The amount of extract typically used in transcription reactions (20 μ l) contains 5–10 ng of UBF. This UBF can be removed efficiently from transcription extracts by immunodepletion (Cairns and McStay, 1995). Full transcription activity can be restored by the addition of recombinant xUBF. Thus, immunodepletion of xUBF does not result in co-precipitation of any other essential factor. We have demonstrated previously that UBF makes protein-protein contacts with Rib1 (Bodeker *et al.*, 1996). These interactions do not occur in the presence of the α -UBF polyclonal antisera used in this immunodepletion. Thus Rib1 does not co-precipitate with UBF. We have used this depleted extract to test enhancer function over a wide range of xUBF concentrations, starting from amounts lower than that normally found in the transcription extract and increasing to ~50 times the level observed in the non-depleted extract (Figure 1). The xUBF used in these experiments has been produced in a baculovirus expression system and has the same specific activity in transcription as purified xUBF (see Materials and methods for details). Promoter-only templates (pGem40 and pGem52, Figure 1A), in competition with each other, are transcribed with equal efficiency at every xUBF input tested (Figure 1B). Promoter plus enhancer (pGem40EX, Figure 1A) and promoter-only (pGem52) templates in competition are also transcribed with equal efficiency at low xUBF inputs (0–10 ng). However, at higher xUBF inputs (50–500 ng), pGem40EX

progressively out-competes pGem52 (Figure 1B). Quantitation of these transcription signals shows that at an input of 500 ng of xUBF, an enhancer-containing template is 26-fold more transcriptionally active than a promoter-only template (Figure 1C). This level of enhancer function is as high, if not higher, than levels observed *in vivo* (Busby and Reeder, 1983; Moss, 1983; Reeder *et al.*, 1983; Labhart and Reeder, 1984, 1985). Thus it appears that the inability of S100 extracts to support enhancement is principally a function of UBF concentration. We have also shown that untreated S100 extract supplemented with baculovirus-produced xUBF supports enhancement (data not shown). We believe that the role of UBF in enhancement is direct and involves the interaction of UBF with enhancer DNA. This is consistent with the observation that enhancers can compete for transcription initiation *in trans* and that this effect can be rescued by the addition of excess UBF (Pikaard *et al.*, 1989). It is worth noting that at low UBF inputs in the experiment described above (0–10 ng), enhancers appear to titrate UBF from promoters both *in cis* and *trans*. (Figure 1B, compare reactions 3, 5 and 7 with 4, 6 and 8). We believe that the high levels of UBF required to observe enhancer action *in vitro* are a closer reflection of the *in vivo* situation (see Discussion).

Enhancers function *in vitro* in a position- and orientation-independent manner

One of the defining characteristics of enhancer action is that it is position and orientation independent with respect to the promoter (Busby and Reeder, 1983; Reeder *et al.*, 1983; Labhart and Reeder, 1984, 1985). We have compared enhancer function on plasmid templates with enhancers located immediately upstream of the promoter (pGem40EX) or located 1 kb upstream in the reverse orientation with respect to the promoter (pGem40EX*, Figure 1A). pGem40EX and pGem40EX* are transcribed 30- and 20-fold respectively more efficiently than a promoter-only template (pGem52) in competition (Figure 1D). It appears that in the presence of a high concentration of xUBF, *Xenopus* RNA pol I enhancers function similarly to *in vivo*.

Enhancers function by increasing the probability of stable complex formation on a linked promoter

Enhancers potentially could influence a linked promoter in two distinct ways. Enhancers could alter the nature of the transcription complex on the linked promoter resulting in higher levels of transcription initiation from that promoter. Alternatively, they could increase the probability of transcription complex formation on a linked promoter. In order to discriminate between these mechanisms, we have used the *in vitro* system to investigate the timing of enhancer action and the stability of the transcription complexes formed on enhancer-linked and enhancer-minus promoters.

When the enhancer-containing template, pGem40EX, is competed against the promoter-only template, pGem52, we observe a linear increase in transcription signal from each template over the course of the standard 2 h transcription reaction (Figure 2A). In addition, we have calculated that the enhancement varies by at most 2-fold throughout the course of the reaction (Figure 2B). These observations suggest that the enhancer effect is established early in the

Fig. 1. High levels of UBF facilitate RNA pol I enhancer function *in vitro*. **(A)** Structure of plasmid templates. pGem40 and pGem52 contain identical *Xenopus* RNA pol I promoter sequences (open box and cross-hatched box respectively) but differ in linker sequence downstream of +34 such that transcripts from each promoter can be distinguished using an S1 nuclease protection assay (see Materials and methods for details). In pGem40EX, the *Xenopus* sequences extend upstream to position -970 to include a single block of enhancer elements (black boxes). In the plasmid pGem40EX*, a single enhancer block in an inverse orientation is located in vector sequences 1 kb upstream of the pGem40 promoter. **(B)** Enhancers function in the presence of a high xUBF input. Transcription reactions contained 20 μ l of xUBF-depleted extract, 400 ng of template DNA, 20 μ l of transcription buffer and the amount of baculovirus-produced xUBF indicated. Reactions 1, 3, 5, 7, 9, 11 and 13 contained an equimolar mixture of the templates pGem40 and pGem52. Reactions 2, 4, 6, 8, 10, 12 and 14 contained an equimolar mixture of the templates pGem40EX and pGem52. Reactions were probed for transcripts from each promoter type (indicated by an arrow on the right side of the gel). **(C)** Quantitation of enhancer function. Transcription signals from the above experiment were quantitated on a phosphorimager (Bio-Rad). The 'Fold Enhancement' was calculated as the ratio of transcription signal observed from pGem40EX to that from pGem52 in the same reaction. The 'Fold Enhancement' is shown for each amount of xUBF input tested. **(D)** Enhancers function at a distance. The templates pGem40 (reaction 1), pGem40EX (reaction 2) and pGem40EX* (reaction 3) were competed individually against pGem52 in the depleted extract supplemented with 500 ng of baculovirus-produced xUBF.

reaction, possibly during stable transcription complex formation.

A template commitment experiment demonstrates that transcription complexes formed on enhancer-linked and enhancer-minus promoters are indistinguishable in terms of their long-term stability (Figure 3A). Transcription complexes were formed on either promoter-only or enhancer-linked templates by pre-incubation in extract supplemented with 200 ng of xUBF. An equal amount of a second template is added prior to the initiation of transcription by the addition of $MgCl_2$ and nucleotide

triphosphates. In each case, very low levels of transcription are observed from the second template relative to the first. Thus enhancer-linked and enhancer-minus promoters are equally capable of preempting transcription complex formation on a second template throughout the course of the standard reaction.

In a related experiment, enhancer-containing and promoter-only templates (pGem40EX and pGem52 respectively) were pre-incubated separately with extract containing high levels of xUBF (200 ng). After combining equal amounts of each pre-incubation mix, transcription

Fig. 2. Timing of enhancer action. (A) Time course of enhancer action. Transcription reactions contained 200 ng of xUBF and the templates pGem40EX and pGem52 in competition. Reactions were terminated after 15, 30, 60 and 120 min incubations. The transcription signal from each template at each time point was quantitated by phosphorimaging, expressed as pixel density units (PDU) $\times 10^4$ and plotted against time. The open box and open circle represent the transcription signals from pGem40EX and pGem52 respectively. (B) Fold enhancement during time course. The 'Fold enhancement' was calculated as the ratio of transcription signal observed from pGem40EX to that from pGem52 in the same reaction. The 'Fold enhancement' is shown for each point in the time course.

was initiated as before. Using this protocol, we observe equal levels of transcription from both templates (Figure 3B, reaction 2). This is in contrast to the situation when templates are mixed prior to pre-incubation and the standard enhancer effect is observed (Figure 3B, reaction 1). In addition to demonstrating that enhancers function during stable complex formation, this experiment further shows that competition is required to observe enhancer function *in vitro*. The only plausible explanation for these results is that in this system enhancers act by increasing the probability of stable complex formation on a linked promoter. Furthermore, transcription complexes formed on promoters with or without enhancers are transcribed with equal efficiency.

The role of UBF in enhancer function is distinct from that at the promoter

Having demonstrated that enhancers exert an influence on stable complex formation, one can imagine two models to explain the role of UBF in this regard. In the first model, one could envisage that enhancers act by recruiting UBF to the promoter. The second model proposes that UBF has its own distinct function at the enhancer. This function involves long-range effects on stable complex formation at the linked promoter. Note that in this model UBF does not translocate from enhancer sequences to the promoter. The observation that enhancers only function

Fig. 3. Enhancers function during stable complex formation. (A) Stable complex formation on promoter-only and promoter plus enhancer templates. Transcription extract (20 μ l) supplemented with xUBF (200 ng) was incubated with template 1 (400 ng) for 15 min on ice. Template 2 (400 ng) was then added together with 20 μ l of transcription buffer. Transcription reactions were then incubated for 2 h at 25°C. In reaction 1, template 1 is pGem40 and template 2 is pGem52. Reaction 2 is the reciprocal of reaction 1. In reaction 3, template 1 is pGem40EX and template 2 is pGem52. Reaction 4 is the reciprocal of reaction 3. (B) Pre-incubation of templates without competition. Transcription reactions 1 and 2 contained 20 μ l of depleted extract supplemented with 200 ng and contained equimolar pGem40EX and pGem52 (400 ng total). In reaction 1, templates were pre-mixed before pre-incubation in the transcription extract. In reaction 2, the templates were pre-incubated independently with 10 μ l of extract then combined after pre-incubation and before the addition of transcription buffer and incubation at 25°C for 2 h.

in vitro at high UBF concentrations combined with the high concentration of UBF in the nucleolus (see Discussion) argues against the first model. UBF would not be expected to be limiting under these conditions. Here we exploit both the species specificity of UBF function at the promoter and the presence of two forms in mammalian cells to demonstrate conclusively that UBF does indeed have a function in enhancement distinct from that at the promoter.

Xenopus and human UBFs are strikingly similar at the amino acid sequence level (Bachvarov and Moss, 1991; McStay *et al.*, 1991a), the only major difference being the presence of an additional HMG box DNA binding motif (HMG box 4) in hUBF. This similarity is reflected in the ability of UBF to bind with comparable affinities to both promoters and enhancers of a heterologous species (Bell *et al.*, 1989; Pikaard *et al.*, 1989, 1990). Despite this conservation in sequence, xUBF and hUBF are not interchangeable with respect to promoter function (Bell *et al.*, 1989). We have shown recently that the absence or presence of HMG box 4 appears to be the sole determinant of this specificity (Cairns and McStay, 1995). Another feature of UBF is that it occurs in two forms (UBF1 and UBF2) in all mammals studied to date (O'Mahony and Rothblum, 1991). Typically, each form represents 50% of total UBF. These different forms arise as a result of alternative splicing. UBF1 is fully functional in transcription. In UBF2, 37 amino acids are deleted from HMG box 2 rendering it inactive in promoter function (Jantzen

Fig. 4. UBF has a role in enhancement distinct from that at the promoter. (A) SDS-polyacrylamide gel of baculovirus-produced UBFs. Three μ g each of baculovirus-produced xUBF, hUBF1 and hUBF2 was electrophoresed on a 7% SDS-polyacrylamide gel and stained with Coomassie blue. Molecular size standards in kilodaltons are indicated on the left. (B) Species specificity of UBF at the promoter. The plasmid pGem40 was transcribed in non-depleted extract (lane 1), UBF-depleted extract (lanes 2, 6 and 10), depleted extract supplemented with 50, 100 or 200 ng of xUBF (lanes 3–5), 50, 100 or 200 ng of hUBF1 (lanes 7–9) and 50, 100 or 200 ng of hUBF2 (lanes 11–13). (C) hUBF1 and hUBF2 support *Xenopus* enhancement. Transcription reactions contained 20 μ l of UBF-depleted extract, 400 ng of template DNA and baculovirus-produced UBF. Reactions 1, 3, 5 and 7 contained an equimolar mixture of the templates pGem40 and pGem52. Reactions 2, 4, 6 and 8 contained an equimolar mixture of the templates pGem40EX and pGem52. The amount and type of baculovirus-produced UBF that supplemented each transcription reaction is shown above.

et al., 1992; Kuhn *et al.*, 1994). The *in vivo* role of UBF2 has remained a puzzle.

Recombinant hUBF1 and hUBF2 were produced in a baculovirus expression system (Figure 4A) (see Materials and methods for details). These UBFs were compared with xUBF for function at the *Xenopus* promoter (Figure 4B). Curiously, hUBF1 appears to have some limited promoter activity in this experiment. This is a consequence of the high level of input. At lower input amounts (5–20 ng), hUBF1 has no measurable activity (our unpublished observation). hUBF2 is inactive in *Xenopus* promoter function at every input amount tested.

hUBF1 and 2 are both incapable of exerting a dominant-negative effect on the function of xUBF at the promoter. Even in the presence of 200 ng of hUBF1 or hUBF2, 5 ng of xUBF is sufficient to confer full promoter activity (Figure 4C, compare reactions 1, 5 and 7). We presume this is a consequence of the high off rate of UBF from promoter sequences in the absence of Rib1 (Putnam and Pikaard, 1992). This observation allowed us to test specifically the ability of hUBF1 and 2 to function in the enhancement while maintaining promoter function with a low amount of xUBF. At low xUBF input (5 ng), enhancer-plus and enhancer-minus templates (pGem40EX and pGem52 respectively) are transcribed with equal efficiency (Figure 4D, reaction 2). At high xUBF input (200 ng), enhancer-plus templates are transcribed 17-fold more efficiently than promoter-only templates (Figure 4D, reaction 4). When a low input of xUBF (5 ng, sufficient to support promoter function) is supplemented with a high input of either hUBF1 (200 ng) or hUBF2 (200 ng), enhancer function is maintained; 70- and 16-fold enhancement

respectively (Figure 4D, reactions 6 and 8). It should be pointed out that hUBF2, unlike hUBF1, does not stimulate total transcription over that observed with 5 ng of xUBF alone. However, the competitive advantage conferred by enhancers *in cis* is still observed. These experiments demonstrate that forms of UBF that cannot function at the promoter can nonetheless function in transcriptional enhancement, and provide conclusive evidence that enhancer sequences do not function by recruiting UBF to the promoter. Instead, we conclude that UBF has a distinct function at the enhancer that must involve long-range effects on promoter activity. A final and important conclusion from this experiment is that we have identified a potential role for mammalian UBF2 *in vivo*, that is transcriptional enhancement.

Enhancer function is flexible with respect to the species of enhancer element and UBF

The observation that hUBF1 and 2 can support the enhancement of *Xenopus* RNA pol I transcription suggests that there are less constraints on UBF in enhancer than promoter function. This point is underscored further by the observation that mouse enhancers function in *Xenopus* oocytes and *Xenopus* enhancers in mouse cells (Kuhn *et al.*, 1990; Pikaard *et al.*, 1990). To address this point further, we have investigated mouse enhancer function in the *Xenopus in vitro* system. Promoters with linked *Xenopus* or mouse enhancers (pGem40EX and pGem40EM respectively) were tested in competition with a promoter-only template (pGem52) in the immunodepleted extract supplemented with 200 ng of xUBF (Figure 5A). In this experiment, 21- and 15-fold enhancement was

Fig. 5. Enhancer function is non-species specific. (A) xUBF functions on mouse enhancers. Transcription reactions contained 200 ng of baculovirus-produced xUBF and the templates pGem52 in competition with pGem40 (reaction 1), pGem40EX (reaction 2) or pGem40EM (lane 3). The plasmid pGem40EM contains mouse enhancers cloned upstream of the pGem40 promoter (see Materials and methods for details). (B) hUBF1 and hUBF2 support mouse enhancer function. Transcription reactions 1, 3 and 5 contained an equimolar mixture of the templates pGem40 and pGem52. Reactions 2, 4 and 6 contained an equimolar mixture of the templates pGem40EM and pGem52. The amount and type of baculovirus-produced UBF that supplemented each transcription reaction is shown above.

observed with *Xenopus* and mouse enhancers respectively. hUBF1 and 2 were then compared with xUBF in their ability to enhance transcription from a *Xenopus* promoter linked to mouse enhancers (Figure 5B). The templates pGem40EM and pGem52 were transcribed in competition in immunodepleted extract supplemented with 200 ng of xUBF (Figure 5B, reactions 1 and 2), 5 ng of xUBF plus 200 ng of hUBF1 (Figure 5B, reactions 3 and 4) or 5 ng of xUBF plus 200 ng of hUBF2 (Figure 5B, reactions 5 and 6). This experiment demonstrates that hUBF1 and 2 can indeed function in the enhancement of transcription from a *Xenopus* RNA pol I promoter by linked mouse enhancers elements. Thus we can conclude that RNA pol I transcriptional enhancement is flexible with respect to both the species of enhancer element and the UBF employed. These observations suggest that there is a fundamental difference between the functions of UBF at the enhancer and at the promoter. This point will be addressed in the Discussion. UBF has been demonstrated to bind to both mouse and *Xenopus* enhancer sequences despite any obvious sequence homology (Pikaard *et al.*, 1990). Therefore, the observation that mouse enhancer elements function in the *Xenopus in vitro* system strengthens the connection between UBF binding to enhancer sequences and enhancer function.

Rib1 can interact with a UBF–enhancer complex

In the preceding experiments, we have demonstrated that enhancers increase the probability of stable complex formation on a linked promoter. The most likely mechanism is that enhancers act by recruiting some component

Fig. 6. Rib1 interacts with an enhancer–UBF complex. (A) Rib1 binds to enhancers in the presence of xUBF. Transcription reactions contain 10 μ l of heparin 0.4 M fraction combined with 10 μ l of a Rib1 fraction (lane 1), 10 μ l of CB100 (lane 2), 10 μ l of eluate from an enhancer affinity column that had been pre-loaded with xUBF (lane 3) or 10 μ l of eluate from an enhancer affinity column in the absence of xUBF (lane 4). (B) Rib1 binds to enhancers in the presence of hUBF1 and 2. Transcription reactions contain 10 μ l of heparin 0.4 M fraction combined with 10 μ l of CB100 (lane 1), 10 μ l of eluate from an enhancer affinity column that had been pre-loaded with xUBF (lane 2), 10 μ l of eluate from an enhancer affinity column that had been pre-loaded with hUBF1 (lane 3), 10 μ l of eluate from an enhancer affinity column that had been pre-loaded with hUBF2 (lane 4) or 10 μ l of eluate from an enhancer affinity column in the absence of UBF (lane 5).

of the stable transcription complex to the promoter. We have demonstrated that enhancers do not act by recruiting UBF to the promoter. As Rib1 is the other component of the stable transcription complex (McStay *et al.*, 1991a), we suggest that the enhancer–UBF complex recruits Rib1 to the promoter. A prediction of this model is that Rib1 can interact with an enhancer–UBF complex. Consistent with this, we have demonstrated recently that UBF can make multiple protein contacts with Rib1 in a DNA-independent manner (Bodeker *et al.*, 1996). It is entirely possible, however, that once bound to enhancer DNA these interacting surfaces of UBF are unavailable for interaction with Rib1. In order to test whether Rib1 can interact with enhancer-bound UBF, we have prepared a DNA affinity column that contains *Xenopus* enhancer sequences covalently linked to Sepharose beads. This column was pre-loaded with xUBF, then a fraction from a heparin–Sepharose column that contains Rib1 activity was applied. We have demonstrated previously that Rib1 is the only activity present in this heparin fraction that is required for RNA pol I transcription (Bodeker *et al.*, 1996). Bound proteins were eluted subsequently from the enhancer–UBF column and tested for Rib1 activity by combining them with a heparin 0.4 M fraction that contains both UBF and RNA pol I. Using this protocol, we demonstrate that Rib1 can bind to an enhancer–UBF complex (Figure 6A, compare lanes 2 and 3). In a control experiment, we show that no Rib1 activity is recovered from the enhancer–Sepharose column in the absence of xUBF (lane 4). Thus we can conclude that Rib1 can bind to an enhancer–UBF complex but not to enhancer sequences alone. As predicted by the observation that hUBF1 and hUBF2 can function in the enhancement of a

Xenopus promoter (Figure 4C), Rib1 can also bind to complexes of enhancers with hUBF1 and 2 (Figure 6B).

Discussion

High concentrations of UBF facilitate enhancer action *in vitro*

Here we have described a *Xenopus in vitro* system in which enhancers function in many respects as they do *in vivo*. The key feature of this system is that a high concentration of UBF is required for enhancer function. This result is not unexpected, especially when one considers the abundance and subcellular localization of UBF *in vivo*. Quantitative Western blotting has shown that a *Xenopus* culture cell contains 5×10^5 – 10^6 molecules of UBF (C. Cairns and B. McStay, unpublished observation). Immunolocalization experiments show that the majority of this UBF is localized to the nucleolus (Jantzen *et al.*, 1990; Chan *et al.*, 1991). This results in a very high local concentration of UBF *in vivo*. It is estimated that in *Xenopus* there are 500 repeats of the ribosomal gene per haploid genome, with one gene promoter and on average two spacer promoters per repeat. We can calculate, therefore, that there is between a 150- and a 300-fold molar excess of UBF to promoters *in vivo*. We observe enhancer function *in vitro* with only a 5- to 30-fold molar excess of UBF over plasmid templates.

Enhancers function during stable complex formation

An examination of the time course of enhancer action reveals that the enhancer effect is established early in the transcription process. These *in vitro* results are in agreement with *in vivo* observations. Enhancers can function *in trans* in oocytes when catenated with a promoter-only template (Dunaway and Droge, 1989). Since catenated plasmids are resolved almost immediately following microinjection, one can conclude that enhancers act early *in vivo*.

Template commitment experiments demonstrate no measurable difference in the stability of transcription complexes formed on promoters with or without linked enhancers. In addition, when stable complexes are pre-formed independently on enhancer-containing and promoter-only templates, equivalent levels of transcription are observed from each template when they subsequently are co-transcribed. The most reasonable interpretation of these results is that enhancers act by increasing the probability of stable transcription complex formation rather than by increasing the transcription rate from the linked promoter. An electron microscopy-based analysis of transcription complexes on templates injected into *Xenopus* oocytes also indicates that enhancers act by increasing the probability of complex formation on a linked promoter *in vivo* (Oshein *et al.*, 1996). Furthermore, it was demonstrated that enhancers do not alter the re-initiation rate of transcription from a promoter *in cis*. It remains to be seen if enhancers in other RNA pol I systems behave in a similar manner. However, the observation that *Xenopus* and mouse enhancers function across species suggests that this may be generally applicable at least in vertebrates. Recent work on the mechanism of action of RNA pol II enhancers *in vivo* has come to a similar

conclusion. Mammalian cell transfection experiments have been used to demonstrate that enhancers act by increasing the probability of transcription from a linked promoter rather than the rate of transcription from that promoter (Walters *et al.*, 1995, 1996).

UBF has a role in enhancer action distinct from that at the promoter

Since UBF and Rib1 are required for stable transcription complex formation on the *Xenopus* promoter (McStay *et al.*, 1991a), it seems likely that enhancers act by recruiting one or other of these factors. Previously, it has been suggested that enhancers act by recruiting limiting amounts of UBF to the promoter (Pikaard *et al.*, 1989). This could arise by UBF transiently interacting with enhancer sequences then translocating to the promoter. The observation that enhancers only function *in vitro* when UBF is non-limiting for promoter function argues against this model. Alternatively, one could imagine that multimerization of UBF over enhancer sequences may have a synergistic effect on UBF binding at the adjacent promoter. However, the observation that enhancers function when separated from the promoter by 1 kb of vector sequences argues against this model. Another argument against this model is the observation that an enhancer-containing plasmid supplied *in trans* but catenated with a promoter-only plasmid can support enhancer function *in vivo* (Dunaway and Droge, 1989). However, the most persuasive argument against models where enhancers recruit UBF to the promoter is that forms of UBF that do not function at the *Xenopus* promoter, hUBF1 and 2, can nonetheless function in enhancement. From this experiment, we conclude that the role of UBF in enhancement is distinct from that at the promoter. We can also infer from this that enhancer function requires simultaneous and independent loading of promoter and enhancer elements with UBF. Consequently, high levels of UBF are required to facilitate the occupancy of both enhancer and promoter elements on a significant fraction of template molecules.

The role of UBF in enhancer action

How does enhancer-bound UBF increase the probability of stable transcription complex formation at a linked promoter? At this point, it is worth considering further recent work relating to the mechanism of action of RNA pol II transcriptional enhancers. RNA pol II transcriptional enhancers typically contain multiple binding sites for an array of sequence-specific DNA binding proteins. These DNA binding proteins have activation domains that interact with components of the RNA pol II basic transcription machinery, the major target being transcription factor IID (TFIID) (Tjian and Maniatis, 1994). TFIID is comprised of TBP and eight or more TAF_{II}s. Different activation domains contact different components of TFIID (Goodrich and Tjian, 1994). Current evidence suggests that RNA pol II enhancers function by recruiting TFIID to the promoter and that this is mediated by the combined interactions of an array of activation domains with a subset of TAF_{II}s (Sauer *et al.*, 1995a,b). An additional complication has been identified from studies of the T-cell receptor α and the human β interferon gene enhancers, where HMG box proteins have been identified as essential components of

enhancer function (Thanos and Maniatis, 1992; Du *et al.*, 1993; Giese *et al.*, 1995). These HMG box proteins perform an 'architectural' role in enhancer function (Grosschedl *et al.*, 1994). RNA pol II enhancer function therefore requires both 'architectural' components and components with affinity for an element of the basic transcription machinery.

Like RNA pol II enhancer binding factors, UBF has been demonstrated to interact with another key component of the basic RNA pol I transcription machinery, SL1 (Beckman *et al.*, 1995) or Rib1 (Bodeker *et al.*, 1996). SL1 in mammals and Rib1 in *Xenopus* are the RNA pol I equivalent of TFIID. UBF makes protein-protein contacts with the TAF₄₈ and TBP components of SL1 (Kwon and Green, 1994; Beckman *et al.*, 1995). A likely mechanism of enhancer action therefore is that an enhancer-UBF complex recruits Rib1 to the promoter. Consistent with this model, we have demonstrated that Rib1 can interact with an enhancer-UBF complex. We presume that this interaction is mediated through protein-protein contacts with UBF since no binding to enhancer sequences is observed in the absence of UBF. Indeed, we have already demonstrated that UBF makes multiple protein contacts with Rib1 in solution (Bodeker *et al.*, 1996). This raises the question as to why high levels of UBF present in these transcription reactions do not repress transcription by titrating out Rib1. We presume that this protein-protein interaction in solution is weaker than that between Rib1 and the promoter-UBF complex or indeed the enhancer-UBF complex.

As discussed above, UBF has been described as an architectural transcription factor (Reeder *et al.*, 1995). It is interesting to speculate that RNA pol I transcriptional enhancement has an 'architectural' component and that this is mediated by UBF. This proposed architectural role for UBF in enhancement must be fundamentally different from that at the promoter. Enhancer function appears to be flexible with respect to the species of enhancer sequence or UBF. Promoter function of UBF is exquisitely sensitive to the species of UBF employed (Bell *et al.*, 1989; Cairns and McStay, 1995). One possible explanation for this difference is that Rib1 interacts with enhancers by protein-protein contacts but with the promoter by a combination of protein-protein and protein-DNA interactions, as has been demonstrated for SL1 (Bell *et al.*, 1988; Beckman *et al.*, 1995).

Concluding remarks

We realize that all of the experiments described here were performed in crude extracts and that there is the potential for other, as yet unidentified factors to be involved in enhancer function. However, this does not alter the principal conclusion of this work, namely that UBF has a role in enhancement distinct from that at the promoter. The ability of UBF to perform these multiple roles in RNA pol I transcription should not be surprising, especially when one considers that it is a transcription factor dedicated to the synthesis of a single essential gene product.

Materials and methods

Plasmid constructs

The plasmids pGem40 and pGem52 were constructed by cloning the -245/+50 *SalI*-*Bam*HI fragment of ψ 40 and the -245/+62 *SalI*-*Bam*HI

fragment of ψ 52 respectively (Labhart and Reeder, 1984) into the *SalI* and *Bam*HI sites of the vector pGem3 (Promega). pGem40EX was constructed by cloning a -970/+50 *SalI*-*Bam*HI fragment of pXlr401 (Labhart and Reeder, 1984) into the *SalI* and *Bam*HI sites of the vector pGem3. In the plasmid pGem40EX*, a single enhancer block (-970/-245) was subcloned from pGem40EX as a *SmaI*-*HincII* fragment into the unique *SspI* site present in the vector sequences of the plasmid pGem40. In the resulting plasmid, the enhancer block is in a reverse orientation with respect to, and 1 kb upstream of, the promoter. The plasmid pMrE13 contains the mouse ribosomal gene intergenic spacer sequences -1930/-169 cloned as a *SalI* fragment into the vector pUC9 (Kuhn *et al.*, 1990). This entire insert was subcloned as a *HindIII*-*SalI* partially digested fragment into the *HindIII* and *SalI* sites of the plasmid pGem40. The resulting plasmid is called pGem40EM. The plasmid pGemEX(8) contains eight blocks of enhancer elements (-970/-245) in a tandem array, cloned as a *SalI*-*XhoI* fragment into the *SalI* and *XhoI* sites of a modified pGem3 vector.

Transcription extracts and assays

S100 transcription extracts in CB100 [25 mM HEPES pH 7.9, 100 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 20% glycerol and protease inhibitors] were prepared from the *Xenopus laevis* cell line, XIK2, as described previously (McStay and Reeder, 1990). Immunodepletion of UBF from S100 extract has been described elsewhere (Cairns and McStay, 1995). Briefly, S100 extract (5.0 ml) was incubated with 50 μ l of α -xUBF antiserum on ice for 30 min then chromatographed three times over a 1.0 ml protein A-Sepharose Fast Flow column (Pharmacia). Immunodepletion was >95% efficient as determined by Western blotting.

In some experiments, fractionated extracts were employed. Heparin 0.4 M and Rib1 fractions have been described previously (McStay *et al.*, 1991a). The heparin 0.4 M fraction elutes from heparin-Sepharose with 400 mM KCl and contains both RNA pol I activity and UBF. The Rib1 fraction elutes from heparin-Sepharose with 600 mM KCl and contains no detectable RNA pol I activity or UBF.

In transcription reactions, immunodepleted extract (20 μ l) was combined with baculovirus-expressed UBF (in 1 μ l volume) and incubated with template DNA (400 ng total) for 10 min on ice. Reactions were initiated by the addition of 20 μ l of transcription buffer (25 mM HEPES pH 7.9, 80 mM KCl, 12 mM MgCl₂, 10 mM creatine phosphate, 1 mM DTT, 100 μ g/ml α -amanitin, 1 mM nucleotide triphosphates) and incubation at 25°C. The final reaction conditions were: 25 mM HEPES pH 7.5, 90 mM KCl, 6 mM MgCl₂, 10% glycerol, 1 mM DTT, 5 mM creatine phosphate, 50 μ g/ml α -amanitin, 1 U/ μ l RNasin (Promega), 0.5 mM NTPs and 10 μ g/ml template DNA. Unless otherwise stated, reactions were allowed to proceed for 2 h. Reactions were terminated and transcripts were detected using S1 nuclease protection with a probe prepared from pGem40 to detect transcripts from pGem40, pGem40EX, pGem40EX* and pGem40EM and a probe prepared from pGem52 to detect transcripts from that template (Labhart and Reeder, 1986). Note that in reactions containing templates in competition, the reaction was split and probed independently for transcripts from each promoter type. Transcription signals were quantitated by Phosphorimaging using a GS-250 Molecular Imager (Bio-Rad).

Baculoviral expression of UBF

Full-length xUBF cDNA was cloned into the baculovirus transfer vector pBluebac (Invitrogen) as described previously (Hu *et al.*, 1994). Full-length hUBF1 and 2 cDNAs were cloned as *Bam*HI-*Eco*RI fragments into the *Bam*HI and *Eco*RI sites in the transfer vector pVL1393 (Invitrogen). Recombinant virus was produced for each cDNA clone using the Baculogold transfection system (Pharmingen). For large-scale protein production, Sf9 cells (100-500 ml in spinner culture) were harvested 3 days post-infection and nuclear extracts were prepared as previously described (Dignam *et al.*, 1983). Nuclear extracts were chromatographed successively over BioRex 70 (Bio-Rad) and Mono Q (Pharmacia) ion exchange columns, where UBF elutes with 500 and 450 mM KCl respectively. At this point, recombinant UBF was >95% pure as judged by Coomassie staining of SDS-polyacrylamide gels (see Figure 4A).

Enhancer affinity chromatography

An enhancer DNA affinity column was prepared by digesting pGemEX with *HindIII* and covalently binding it to CNBr-activated Sepharose CL2B as previously described (Kadonaga *et al.*, 1986). The final DNA concentration on the affinity resin was 80 μ g/ml. A Rib1 fraction (250 μ l) was loaded onto enhancer-Sepharose columns (100 μ l) that had been

pre-loaded or not with baculovirus-expressed UBF (10 µg). Columns were washed repeatedly with CB100. Bound proteins were eluted with CB800 (CB100 with 800 mM KCl) and dialysed against CB100.

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References

- Bachvarov,D. and Moss,T. (1991) The RNA polymerase I transcription factor xUBF contains 5 tandemly repeated HMG homology boxes. *Nucleic Acids Res.*, **19**, 2331–2335.
- Bazett-Jones,D.P., Leblanc,B., Herfort,M. and Moss,T. (1994) Short-range DNA looping by the *Xenopus* HMG-box transcription factor, xUBF. *Science*, **264**, 1134–1137.
- Beckman,H., Chen,J.-L., O'Brien,T. and Tjian,R. (1995) Coactivator and promoter-selective properties of RNA polymerase I TAFs. *Science*, **270**, 1506–1509.
- Bell,S.P., Learned,R.M., Jantzen,H.-M. and Tjian,R. (1988) Functional cooperativity between transcription factors UBF1 and SL1 mediates human ribosomal RNA synthesis. *Science*, **241**, 1192–1197.
- Bell,S.P., Pikaard,C.S., Reeder,R.H. and Tjian,R. (1989) Molecular mechanisms governing species-specific transcription of ribosomal RNA. *Cell*, **59**, 489–497.
- Bodeker,M., Cairns,C. and McStay,B. (1996) Upstream binding factor stabilises Rib1, the TATA-binding-protein-containing *Xenopus laevis* RNA polymerase I transcription factor, by multiple protein interactions in a DNA-independent manner. *Mol. Cell. Biol.*, **16**, 5572–5578.
- Busby,S.J. and Reeder,R.H. (1983) Spacer sequences regulate transcription of ribosomal gene plasmids injected into *Xenopus* embryos. *Cell*, **34**, 989–996.
- Cairns,C. and McStay,B. (1995) HMG box 4 is the principal determinant of species specificity in the RNA polymerase I transcription factor UBF. *Nucleic Acids Res.*, **23**, 4583–4590.
- Chan,E.K., Imai,H., Hamel,J.C. and Tan,E.M. (1991) Human autoantibody to RNA polymerase I transcription factor hUBF. Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal RNA transcription upstream binding factor. *J. Exp. Med.*, **174**, 1239–1244.
- Comai,L., Tanese,N. and Tjian,R. (1992) The TATA-binding protein and associated factors are integral components of the RNA polymerase I transcription factor, SL1. *Cell*, **68**, 965–976.
- Comai,L., Zomerdijk,J.C., Beckman,H., Zhou,S., Admon,A. and Tjian,R. (1994) Reconstitution of transcription factor SL1: exclusive binding of TBP by SL1 or TFIID subunits. *Science*, **266**, 1966–1972.
- Dignam,J.D., Martin,P.L., Shastry,B.S. and Roeder,R.G. (1983) Eukaryotic gene transcription with purified components. *Methods Enzymol.*, **101**, 582–598.
- Du,W., Thanos,D. and Maniatis,T. (1993) Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. *Cell*, **74**, 887–898.
- Dunaway,M. and Droge,P. (1989) Transactivation of the *Xenopus* rRNA gene promoter by its enhancer. *Nature*, **341**, 657–659.
- Eberhard,D., Tora,L., Egly,J.M. and Grummt,I. (1993) A TBP-containing multiprotein complex (TIF-IB) mediates transcription specificity of murine RNA polymerase I. *Nucleic Acids Res.*, **21**, 4180–4186.
- Giese,K., Kingsley,C., Kirshner,J.R. and Grosschedl,R. (1995) Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein–protein interactions. *Genes Dev.*, **9**, 995–1008.
- Goodrich,J.A. and Tjian,R. (1994) TBP–TAF complexes: selectivity factors for eukaryotic transcription. *Curr. Opin. Cell Biol.*, **6**, 403–409.
- Grosschedl,R., Giese,K. and Pagel,J. (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. *Trends Genet.*, **10**, 94–100.
- Hu,C.H., McStay,B., Jeong,S.W. and Reeder,R.H. (1994) xUBF, an RNA polymerase I transcription factor, binds crossover DNA with low sequence specificity. *Mol. Cell. Biol.*, **14**, 2871–2882.
- Jantzen,H.M., Admon,A., Bell,S.P. and Tjian,R. (1990) Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature*, **344**, 830–836.
- Jantzen,H.M., Chow,A.M., King,D.S. and Tjian,R. (1992) Multiple domains of the RNA polymerase I activator hUBF interact with the TATA-binding protein complex hSL1 to mediate transcription. *Genes Dev.*, **6**, 1950–1963.
- Kadonaga,J.T. and Tjian,R. (1986) Affinity purification of sequence-specific DNA binding proteins. *Proc. Natl Acad. Sci. USA*, **83**, 5889–5893.
- Kuhn,A., Deppert,U. and Grummt,I. (1990) A 140-base-pair repetitive sequence element in the mouse rRNA gene spacer enhances transcription by RNA polymerase I in a cell-free system. *Proc. Natl Acad. Sci. USA*, **87**, 7527–7531.
- Kuhn,A., Voit,R., Stefanovsky,V., Evers,R., Bianchi,M. and Grummt,I. (1994) Functional differences between the two splice variants of the nucleolar transcription factor UBF: the second HMG box determines specificity of DNA binding and transcriptional activity. *EMBO J.*, **13**, 416–424.
- Kwon,H. and Green,M.R. (1994) The RNA polymerase I transcription factor, upstream binding factor, interacts directly with the TATA box-binding protein. *J. Biol. Chem.*, **269**, 30140–30146.
- Labhart,P. and Reeder,R.H. (1984) Enhancer-like properties of the 60/81 bp elements in the ribosomal gene spacer of *Xenopus laevis*. *Cell*, **37**, 285–289.
- Labhart,P. and Reeder,R.H. (1985) *Xenopus* ribosomal gene enhancers function when inserted inside the gene they enhance. *Nucleic Acids Res.*, **13**, 8999–9009.
- Labhart,P. and Reeder,R.H. (1986) Characterization of three sites of RNA 3' end formation in the *Xenopus* ribosomal gene spacer. *Cell*, **45**, 431–443.
- McStay,B. and Reeder,R.H. (1986) A termination site for *Xenopus* RNA polymerase I also acts as an element of an adjacent promoter. *Cell*, **47**, 913–920.
- McStay,B. and Reeder,R.H. (1990) A DNA-binding protein is required for termination of transcription by RNA polymerase I in *Xenopus laevis*. *Mol. Cell. Biol.*, **10**, 2793–2800.
- McStay,B., Hu,C.-H., Pikaard,C.S. and Reeder,R.H. (1991a) xUBF and Rib 1 are both required for formation of a stable polymerase I promoter complex in *X.laevis*. *EMBO J.*, **10**, 2297–2303.
- McStay,B., Frazier,M.W. and Reeder,R.H. (1991b) xUBF contains a novel dimerization domain essential for RNA polymerase I transcription. *Genes Dev.*, **5**, 1957–1968.
- Mishima,Y., Financsek,I., Kominami,R. and Muramatsu,M. (1982) Fractionation and reconstitution of factors required for accurate transcription of mammalian ribosomal RNA genes: identification of a species-dependent initiation factor. *Nucleic Acids Res.*, **10**, 6659–6670.
- Moss,T. (1983) A transcriptional function for the repetitive ribosomal spacer in *Xenopus laevis*. *Nature*, **302**, 223–228.
- O'Mahony,D.J. and Rothblum,L.I. (1991) Identification of two forms of the RNA polymerase I transcription factor UBF. *Proc. Natl Acad. Sci. USA*, **88**, 3180–3184.
- Osheim,Y.N., Mougey,E.B., Windle,J., Anderson,M., O'Rielly,M., Miller,O.L., Beyer,A. and Sollner-Webb,B. (1996) Metazoan rDNA enhancer acts by making more genes transcriptionally active. *J. Cell Biol.*, **133**, 943–954.
- Pikaard,C.S. (1994) Ribosomal gene promoter domains can function as artificial enhancers of RNA polymerase I transcription, supporting a promoter origin for natural enhancers in *Xenopus*. *Proc. Natl Acad. Sci. USA*, **91**, 464–468.
- Pikaard,C.S., Pape,L.K., Henderson,S.L., Ryan,K., Paalman,M.H., Lopata,A., Reeder,R.H. and Sollner-Webb,B. (1990) Enhancers for RNA polymerase I in mouse ribosomal DNA. *Mol. Cell. Biol.*, **10**, 4816–4825.
- Putnam,C.D., Copenhaver,G.P., Denton,M.L. and Pikaard,C.S. (1994) The RNA polymerase I transactivator upstream binding factor requires its dimerization domain and high-mobility-group (HMG) box 1 to bend, wrap, and positively supercoil enhancer DNA. *Mol. Cell. Biol.*, **14**, 6476–6488.
- Putnam,C.D. and Pikaard,C.S. (1992) Cooperative binding of the *Xenopus* RNA polymerase I transcription factor xUBF to repetitive ribosomal gene enhancers. *Mol. Cell. Biol.*, **12**, 4970–4980.
- Reeder,R.H. (1992) The regulation of transcription by RNA polymerase I. In McKnight,S.L. and Yamamoto,K. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 315–347.
- Reeder,R.H., Roan,J.G. and Dunaway,M. (1983) Spacer regulation of *Xenopus* ribosomal gene transcription: competition in oocytes. *Cell*, **35**, 449–456.
- Reeder,R.H., Pikaard,C.S. and McStay,B. (1995) UBF, an architectural element for RNA polymerase I promoters. In Eckstein,F. and

- Lilley,D.M.J. (eds), *Nucleic Acids and Molecular Biology*. Springer-Verlag, Berlin, Heidelberg, Vol. 9, pp. 251–263.
- Sauer,F., Hansen,S.K. and Tjian,R. (1995a) Multiple TAFIIIs directing synergistic activation of transcription. *Science*, **270**, 1783–1788.
- Sauer,F., Hansen,S.K. and Tjian,R. (1995b) DNA template and activator–coactivator requirements for transcriptional synergism by *Drosophila* bicoid. *Science*, **270**, 1825–1828.
- Schnapp,A., Clos,J., Hadelt,W., Schreck,R., Cvekl,A. and Grummt,I. (1990) Isolation and functional characterization of TIF-IB, a factor that confers promoter specificity to mouse RNA polymerase I. *Nucleic Acids Res.*, **18**, 1385–1393.
- Schultz,M.C., Choe,S.Y. and Reeder,R.H. (1993) *In vitro* definition of the yeast RNA polymerase I enhancer. *Mol. Cell. Biol.*, **13**, 2644–2654.
- Stefanovsky,V.Y., Bazett-Jones,D.P., Pelletier,G. and Moss,T. (1996) The DNA supercoiling architecture induced by the transcription factor xUBF requires three of its five HMG-boxes. *Nucleic Acids Res.*, **24**, 3208–3215.
- Thanos,D. and Maniatis,T. (1992) The high mobility group protein HMG I(Y) is required for NF-kappa B-dependent virus induction of the human IFN-beta gene. *Cell*, **71**, 777–789.
- Tjian,R. and Maniatis,T. (1994) Transcriptional activation: a complex puzzle with few easy pieces. *Cell*, **77**, 5–8.
- Tower,J., Culotta,V.C. and Sollner-Webb,B. (1986) Factors and nucleotide sequences that direct ribosomal DNA transcription and their relationship to the stable transcription complex. *Mol. Cell. Biol.*, **6**, 3451–3462.
- Walters,M.C., Fiering,S., Eidemiller,J., Magis,W., Groudine,M. and Martin,D.I. (1995) Enhancers increase the probability but not the level of gene expression. *Proc. Natl Acad. Sci. USA*, **92**, 7125–7129.
- Walters,M.C., Magis,W., Fiering,S., Eidemiller,J., Scalzo,D., Groudine,M. and Martin,D.I. (1996) Transcriptional enhancers act in *cis* to suppress position-effect variegation. *Genes Dev.*, **10**, 185–195.
- Zomerdijk,J.C., Beckman,H., Comai,L. and Tjian,R. (1994) Assembly of transcriptionally active RNA polymerase I initiation factor SL1 from recombinant subunits. *Science*, **266**, 2015–2018.

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