The EMBO Journal Vol.17 No.10 pp.2855–2864, 1998

Cloning and functional characterization of PTRF, a novel protein which induces dissociation of paused ternary transcription complexes

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Termination of transcription by RNA polymerase I (Pol I) is a two-step process which involves pausing of elongating transcription complexes and release of both pre-rRNA and Pol I from the template. In mouse, pausing of elongation complexes is mediated by the transcription termination factor TTF-I bound to the 'Sal box' terminator downstream of the rDNA transcription unit. Dissociation of paused ternary complexes requires a cellular factor, termed PTRF for Pol I and transcript release factor. Here we describe the molecular cloning of a cDNA corresponding to murine PTRF. Recombinant PTRF is capable of dissociating ternary Pol I transcription complexes *in vitro* as revealed by release of both Pol I and nascent transcripts from the template. Consistent with its function in transcription termination, PTRF interacts with both TTF-I and Pol I. Moreover, we demonstrate specific binding of PTRF to transcripts containing the 3′ end of pre-rRNA. Substitution of 3′-terminal uridylates by guanine residues abolishes PTRF binding and impairs release activity. The results reveal a network of protein–protein and protein–nucleic acid interactions that governs termination of Pol I transcription.

Keywords: protein interactions/RNA polymerase I/ternary complexes/transcript release/transcription termination

Introduction

Transcription by all three classes of nuclear RNA polymerases proceeds in distinct steps designated initiation, elongation and termination. Although transcription initiation is a major target for regulation, a growing body of evidence indicates that elongation and 3′ end formation also play important roles in modulating cellular transcriptional activity (reviewed in Manley and Proudfoot, 1994; Shilatifard *et al*., 1997). Like all other steps in RNA synthesis, formation of the 3′ end of nascent transcripts is a complex process that requires both protein–nucleic acid and protein–protein interactions. Thus, both pausing of the transcription elongation complex and proper 3′ end formation, i.e. dissociation of the ternary transcription complex and 3′-terminal processing of the primary transcript, are mediated by ancillary proteins which recognize specific sequence motifs or structures within DNA or RNA and are capable of communicating with components of the transcription apparatus to terminate transcription.

While transcription termination of genes transcribed by RNA polymerase II is still poorly characterized, the mechanism of transcription termination by RNA polymerase I (Pol I) is much better understood. In short, termination of Pol I occurs at specific terminator elements downstream of the pre-rRNA coding region (Grummt *et al*., 1985; Bartsch *et al.*, 1987). Despite marked differences in the *cis*-acting elements and *trans*-acting factors in species as diverse as yeast, *Xenopus*, human, rat and mouse, the mechanism of Pol I transcription termination in all eukaryotes is probably very similar (reviewed by Reeder and Lang, 1994, 1997; Mason *et al.*, 1998). All characterized Pol I terminator elements function in only one orientation and are recognized by a specific DNA-binding protein that stops elongating Pol I. The terminator protein, i.e. TTF-I in mammals or Reb1p in yeast, presumably contacts the elongating RNA polymerase and mediates the termination reaction. In addition to the binding site for the terminator protein, an upstream element that codes for the last 10–12 nucleotides of pre-rRNA is required for complete termination, e.g. for release of the terminated transcripts and Pol I.

In the mouse, termination of Pol I transcription occurs 565 bp downstream of the 28S RNA coding region (Grummt *et al*., 1985). The 3′ endpoint of the pre-rRNA maps upstream of T_1, the first of 10 'Sal box' terminator elements (AGGTCGACCAGA/TT/ANTCCG) which are clustered within several hundred base pairs of the non-transcribed spacer downstream of the 28S rRNA coding region (Grummt *et al*., 1986). The individual Sal box elements are flanked by long pyrimidine stretches, not uncommon for a eukaryotic terminator. Indeed, a T-rich element upstream of the first terminator (T_1) has been demonstrated to be required for both efficient transcript release and 3′-terminal processing (Kuhn and Grummt, 1989; Lang and Reeder, 1995; Mason *et al*., 1997a). The overall base composition of the upstream element determines the efficiency of transcript release (Kuhn and Grummt, 1989; Lang and Reeder, 1995).

The availability of cloned terminator proteins facilitated the establishment of cell-free systems which terminate at the same sites utilized *in vivo* and thus allowed functional studies concerning the mechanism of transcription termination. These studies revealed that murine Pol I transcription termination requires two *cis*-acting elements, the Sal box terminator and the T-rich element located upstream of the terminator T_1, as well as two *trans*-acting factors, i.e. TTF-I and a novel activity that dissociates TTF-I-paused transcription complexes (Mason *et al.*, 1997a). This novel activity is now designated PTRF, for Pol I and transcript

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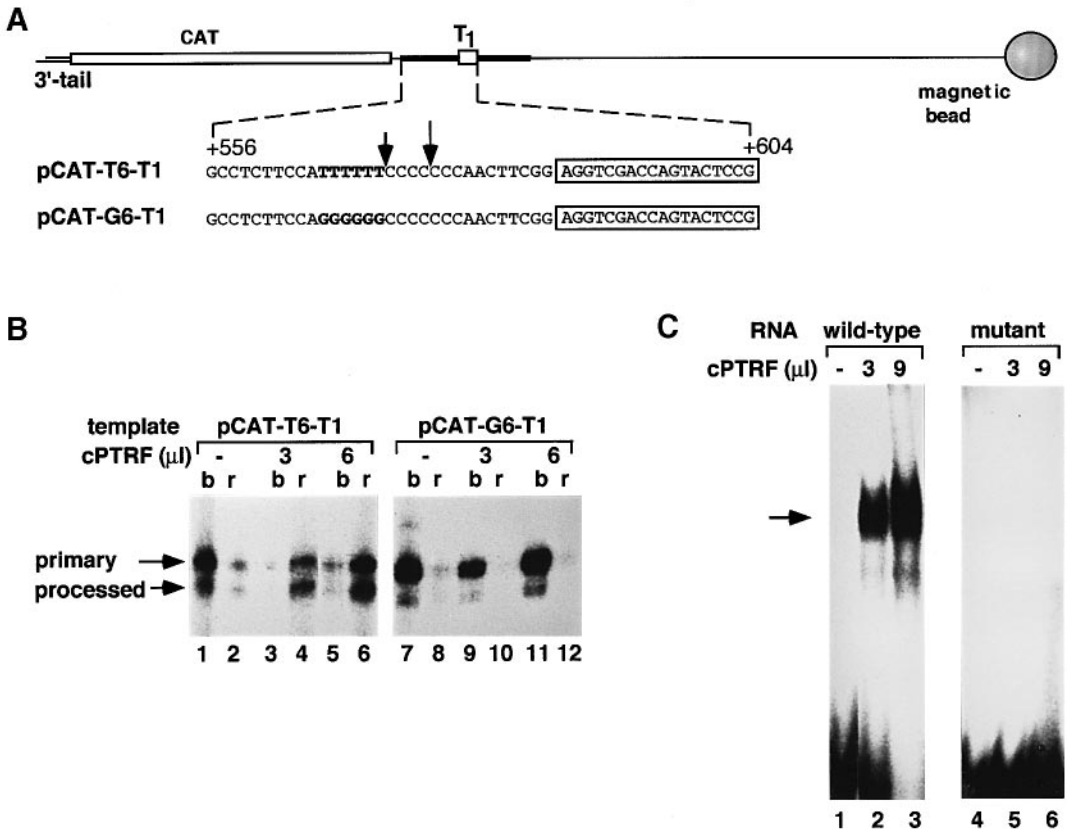

Fig. 1. Functional properties of cellular PTRF. (**A**) Diagram showing the structure of the tailed template pCAT-T6-T1 and the mutant pCAT-G6-T1. The positions of the extended 3′ overhang, the CAT fragment (open box) and the 3′-terminal rDNA fragment (thick line) including the T_1 terminator element are indicated. The nucleotide sequence of the terminator region is shown below. The 18 bp Sal box terminator element is boxed; bold letters mark the six T residues in the flanking region which are substituted by G residues in the mutant pCAT-G6-T1. Numbers indicate the position of nucleotides with respect to the 3′ end of the 28S RNA coding region. The two vertical arrows mark the position of the primary and 3′-terminally processed transcript whose lengths are 202 and 198 nucleotides, respectively. (**B**) PTRF facilitates transcript release. Transcript release was assayed on immobilized tailed templates pCAT-T6-T1 (lanes 1–6) or pCAT-G6-T1 (lanes 7–12). Reactions contained 5 µl of Pol I (0.2 U), 20 ng of TTF-I and 0, 3 or 6 µl of cellular PTRF (MonoS fraction, 0.5 ng of PTRF per µl) as indicated. RNA synthesized during a 10 min incubation was fractionated into template-bound (b) and released (r) transcripts. (**C**) RNA-binding activity of PTRF. ^{32}P-labeled RNA probes containing 20 nucleotides from the 3′-terminus of mouse pre-rRNA (lanes 1–3) were incubated in the absence or presence of cellular PTRF (MonoS fraction) as indicated. In lanes 4–6, a mutant RNA probe was used in which the six U residues were replaced by guanosines. The ribonucleoprotein complexes were separated from unbound RNA by electrophoresis in non-denaturing 5% polyacrylamide gels and visualized by autoradiography.

release factor. PTRF activity was initially identified in partially purified fractions by complementation of a release-deficient cellular Pol I for transcript release. Here we report the cloning and functional characterization of PTRF and demonstrate that the recombinant protein possesses functional properties similar to those of the partially purified cellular factor. Like cellular PTRF, the recombinant factor allows release of nascent Pol I transcripts from ternary transcription complexes that are paused by TTF-I. We demonstrate specific interaction of PTRF with both TTF-I and Pol I, and show that PTRF binds *in vitro* to transcripts containing the 3′ end of pre-rRNA. Based on these properties of PTRF, a model of transcription termination is presented.

Results

PTRF mediates dissociation of ternary transcription complexes paused by TTF-I

The transcript release assay utilizes the template pCAT-T6-T1 which contains part of the chloramphenicol acetyltransferase (*CAT*) gene fused to a fragment from the 3′-terminal spacer of mouse rDNA including one Sal box element and flanking sequences (Figure 1A). A 10 nucleotide single-stranded 3′ extension or 'tail' was added to the 5′ end, which facilitates transcription initiation in the absence of auxiliary factors (Kuhn *et al.*, 1990). Attachment of a magnetic bead to the other end of the template allows separation of ternary elongation complexes, which are paused at the terminator and are still attached to the template, from free Pol I and transcripts which are released into the supernatant.

In transcription assays containing Pol I and TTF-I, two closely spaced transcripts are generated, the longer one representing the primary terminated transcript which is converted into the shorter one by a processing reaction that removes four nucleotides from the 3′ end of the nascent transcript (Kuhn and Grummt, 1989; Mason *et al.*, 1997a). In the absence of a PTRF-containing fraction, the majority of transcripts remained bound to the template (Figure 1B, lanes 1 and 2). The proportion of template-bound versus free transcripts in the supernatant changed when increasing amounts of partially purified PTRF (MonoS fraction) were added. At the highest amount of PTRF added, practically all transcripts were found in the supernatant (Figure 1B, lanes 3–6), indicating that ternary

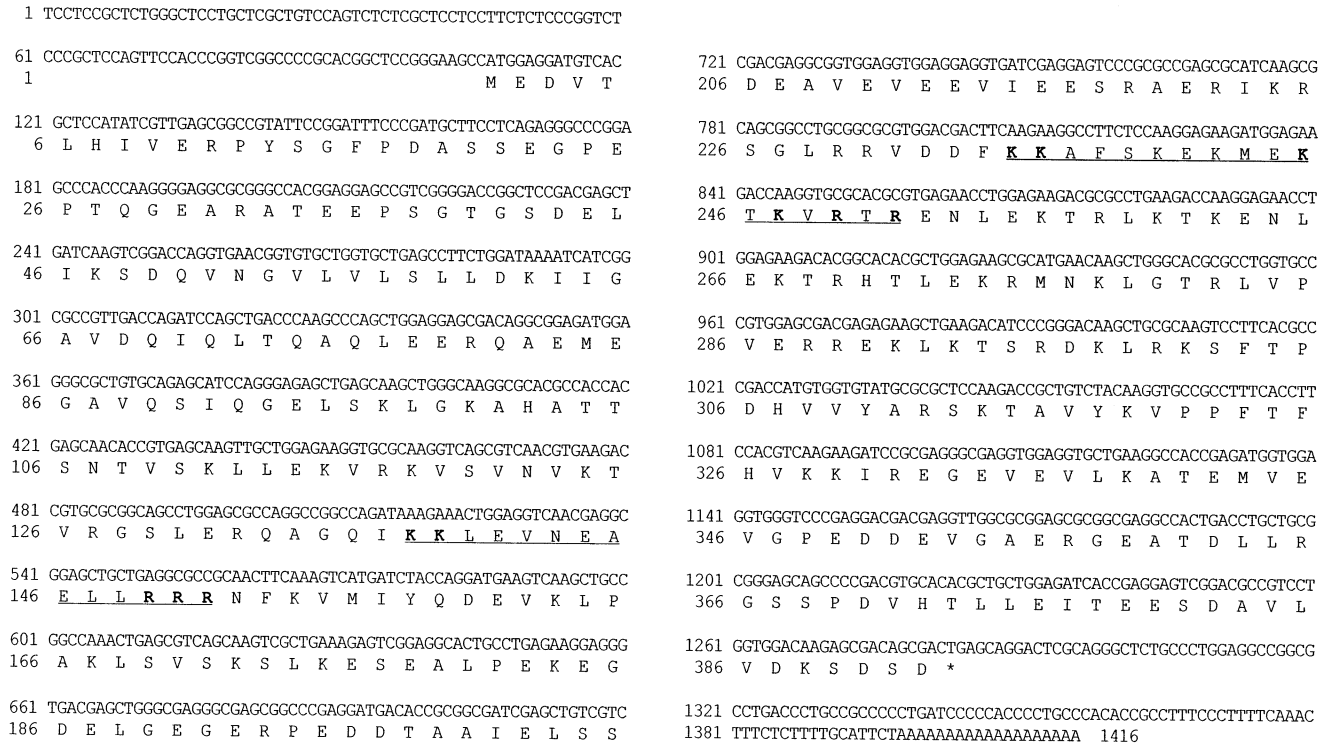

Fig. 2. Nucleotide and deduced amino acid sequence of mouse PTRF. The underlined sequences correspond to two putative nuclear localization signals identified by the PROSITE program (Senger *et al.*, 1995). Two clusters of basic amino acids contained in both bipartite NLSs are marked by bold letters.

complexes were dissociated and the RNA released from the template. Significantly, PTRF function requires DNA sequences upstream of the terminator T_1 which affect the efficiency of 3′ end formation (Kuhn and Grummt, 1989; Lang and Reeder, 1995; Mason *et al*., 1997a). Conversion of the six thymidine residues in the non-template strand (from +566 to +571 with respect to the 3′ end of 28S RNA) into guanosines (pCAT-G6-T1) impairs transcript release (Figure 1B, lanes 7–12). Thus, both PTRF and the T-rich sequence upstream of the terminator T_1 are required for transcript release.

The importance of both the upstream sequence element and PTRF for dissociation of the ternary elongation complex is consistent with previous data demonstrating that Pol I and transcript release depend on sequences contained in the very 3′ end of pre-rRNA. To examine whether PTRF would bind specifically to the end of the primary rDNA transcript, we used T7 RNA polymerase to synthesize a short RNA which contains the same 3′ end as pre-rRNA and therefore resembles the end of the natural Pol I product. In parallel, a mutant transcript was used where the six U residues were converted into guanosines. Binding of cellular PTRF to both wild-type and mutant RNA was measured in an electrophoretic mobility shift assay. As shown in Figure 1C, incubation of PTRF with the wild-type probe (lanes 1–3) yielded a defined complex which was not formed with the mutant RNA probe (lanes 4–6). This result demonstrates that PTRF binds RNA, and the U-rich element is involved in specific PTRF–RNA interaction.

Cloning of PTRF

The strategy for cloning the cDNA encoding PTRF was based on preliminary observations indicating that this factor interacts with TTF-I (unpublished results). We therefore performed a yeast two-hybrid screen (Fields and Song, 1989; Gyuris *et al*., 1993) using TTF-I as a bait. The initial screening of 2×10^7 clones from a human lung fibroblast WI-38 cDNA library yielded five positive clones, one of which encoded a novel protein which was found to represent an N-terminally truncated version of human PTRF (data not shown). The corresponding full-length murine cDNA was obtained by a PCR-based approach as described in Materials and methods. As will be shown below, this cDNA encodes functional murine PTRF. The deduced amino acid sequence of murine PTRF is shown in Figure 2. The cDNA encompasses a 1176 nucleotide open reading frame (ORF) that predicts a 392 amino acid protein with a molecular mass of 44 kDa. The human and mouse sequences are 94% homologous at the amino acid level and contain two putative nuclear localization signals. Both human and mouse PTRF show 89% homology to a chicken protein that has been reported to DDBJ/EMBL/GenBank and is referred to as a putative leucine zipper protein (Sawada *et al*., 1996). The marked sequence homology between the human, mouse and chicken cDNA suggests that PTRF is a highly conserved protein.

Recombinant PTRF interacts with TTF-I

To examine which domain of TTF-I is involved in the interaction with PTRF, a series of N- and C-terminally truncated TTF-I mutants were fused to LexA and transformed into a yeast strain carrying a β-galactosidase reporter and PTRF fused to a transcription activation domain (Figure 3A). In this experiment, the cDNA derived from the initial yeast two-hybrid screen was used which encodes N-terminally truncated human PTRF. The fusion

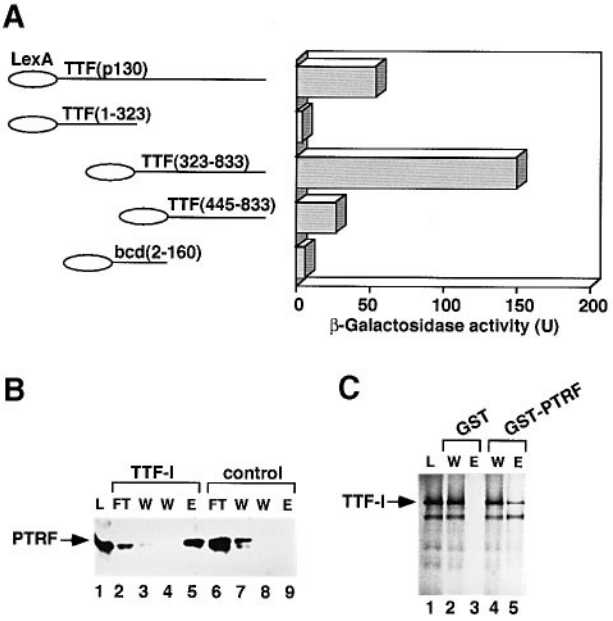

Fig. 3. TTF-I interacts with PTRF *in vivo* and *in vitro*. (**A**) The central part of TTF-I interacts with PTRF. Full-length (TTFp130) and defined regions of murine TTF-I were fused in-frame to the LexA DNA-binding domain and tested in the yeast two-hybrid system for their interaction with PTRF. In this experiment, an N-terminally truncated version of human PTRF, PTRFΔN150, was fused to the B42 transcription activation domain. As a negative control, pRHM1, a plasmid that expresses LexA fused to a transcriptionally inert fragment of the *Drosophila melanogaster* Bicoid protein (amino acid residues 2–160) was used. Numbers refer to the amino acids within TTF-I and Bicoid which are contained in the respective fusion proteins. Activation of the *LacZ* reporter gene was quantified by a liquid β-galactosidase assay. The mean values of three independent experiments are shown. (**B**) PTRF interacts with immobilized TTF-I. Histidine-tagged TTFΔN185 was expressed in baculovirus-infected Sf9 cells (Sander *et al*., 1996) and immobilized on Ni^{2+}-NTA–agarose beads (Pharmacia). A yeast extract (15 μg of total protein) containing HA-tagged PTRFΔN150 (L) was loaded onto a 10 μl column containing bound TTFΔN185 (lanes 2–5) or control Ni^{2+}-NTA beads (lanes 6–9). The flow-through (FT) fraction, 25% of the yeast extract (L), two wash fractions (W) and the total amount of the 1 M KCl eluate (E) were analyzed on Western blots with anti-HA (12CA5) monoclonal antibodies. (**C**) Binding of TTF-I to immobilized PTRF. GST or GST–PTRF were expressed in *E.coli*, bound to glutathione beads, and incubated with ^{35}S-labeled mTTF-I (L). The beads were washed with 10 vols of buffer AM-100 (W) and eluted with high salt buffer AM-1000 (E).

protein containing full-length TTF-I (LexA–TTFp130) reconstitutes the activator required for LacZ expression, resulting in β-galactosidase levels significantly higher than background. Consistent with the N-terminus of TTF-I being dispensable for transcription termination (Evers *et al.*, 1995), no interaction was detected between PTRF and the N-terminal part of TTF-I (TTF1–323). On the other hand, the N-terminal deletion mutant TTFΔN323 which efficiently promotes transcription termination (Evers *et al.*, 1995) produced high levels of β-galactosidase. TTFΔN323-mediated activation of LacZ expression was even higher than that of the full-length protein TTFp130. Thus, in support of previous experiments demonstrating that TTFΔN323 binds to DNA with higher affinity than TTFp130 (Evers *et al*., 1995; Sander *et al*., 1996), the C-terminal half of TTF-I, which harbors the domains involved in DNA-binding and termination activity, also mediates the interaction with PTRF.

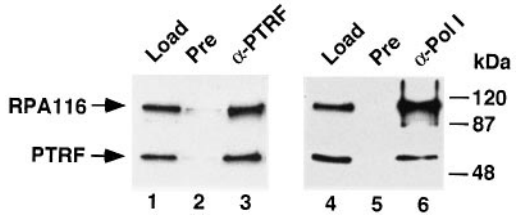

Fig. 4. Co-immunoprecipitation of PTRF and RNA polymerase I. Partially purified nuclear extract proteins (DEAE-280) were incubated with bead-bound rabbit anti-PTRF antibodies (α-PTRF, lane 3), anti-Pol I antibodies (α-PAF/RPA53, lane 6) and the respective pre-immune sera (Pre, lanes 2, 5). Twenty percent of the DEAE-280 fraction (Load) and the total of precipitated proteins were analyzed on immunoblots with anti-PTRF and anti-RPA116 antibodies.

In order to demonstrate that TTF-I and PTRF can also interact *in vitro*, we performed affinity chromatography using either TTF-I or PTRF as immobilized ligands. To monitor binding of PTRF to bead-bound TTF-I, a crude yeast extract containing hemagglutinin (HA)-tagged recombinant PTRF was chromatographed on histidine-tagged TTF-I bound to a nickel-chelate matrix and bound proteins were eluted with salt. The unbound proteins (FT), the wash (W) and the eluate (E) were analyzed on immunoblots using anti-HA antibodies. As shown in Figure 3B, a significant amount of PTRF bound to and could be eluted from the TTF-I beads (lanes 1–5), whereas no binding of PTRF to control beads was observed (lanes 6–9). The reciprocal experiment, i.e. binding of TTF-I to immobilized PTRF, is shown in Figure 3C. A glutathione *S*-transferase (GST) fusion protein containing the entire ORF of murine PTRF (GST–PTRF) was produced in *Escherichia coli*, bound to glutathione–Sepharose beads and used to bind TTF-I which was synthesized in rabbit reticulocyte lysates. Approximately 20% of ^{35}S-labeled TTF-I was retained on the GST–PTRF (Figure 3C, lane 5) but not on the GST control resin (Figure 3C, lane 3). Thus, the *in vitro* binding results confirm the physical interaction between PTRF and TTF-I and extend the observations obtained by the genetic interaction screen in yeast.

PTRF associates with RNA polymerase I

In previous experiments, we have observed a great deal of variability in the extent of transcript release depending on the Pol I preparation used. We have separated two forms of Pol I chromatographically, one that is competent for transcript release on its own, and one that is release-deficient, but can be complemented by cellular fractions containing PTRF (Mason *et al*., 1997a). This result suggests that the release factor is associated with and can be dissociated from Pol I. To monitor the interaction between PTRF and Pol I, antibodies against PTRF and the third largest murine Pol I subunit PAF/RPA53 (Seither *et al*., 1997), respectively, were bound to magnetic beads and incubated with a partially purified protein fraction (DEAE-280) derived from mouse nuclear extracts. Proteins bound to the immobilized antibodies and to control beads, respectively, were analyzed on immunoblots using anti-PTRF and anti-Pol I (α-RPA116) antibodies. As shown in Figure 4, significant amounts of Pol I were co-precipitated with anti-PTRF antibodies (lane 3). In the reciprocal experiment, i.e. co-immunoprecipitation of PTRF with Pol I, we

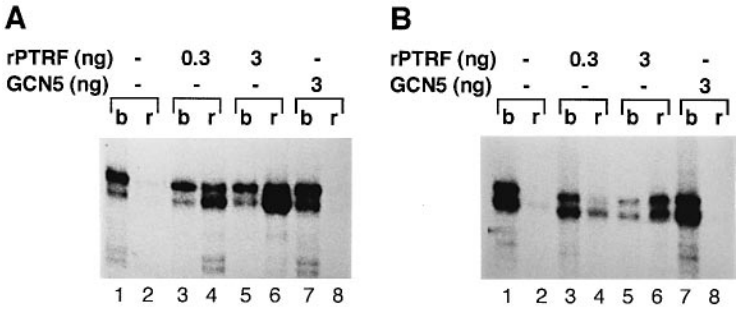

Fig. 5. Recombinant PTRF mediates transcript release. (**A**) Complementation of release-deficient Pol I with recombinant PTRF. Transcript release was assayed in the absence or presence of increasing amounts of recombinant histidine-tagged PTRF or GCN5 as indicated, and the distribution of bound (b) and released (r) transcripts was determined. (**B**) Recombinant PTRF releases transcripts from washed ternary transcription complexes. Transcription reactions were incubated for 5 min to allow Pol I to reach the terminator. The paused complexes were removed by magnetic attraction, washed with buffer AM-200 to remove free Pol I and nucleotides, and incubated for another 5 min with NTPs and recombinant histidine-tagged PTRF or GCN5 as indicated.

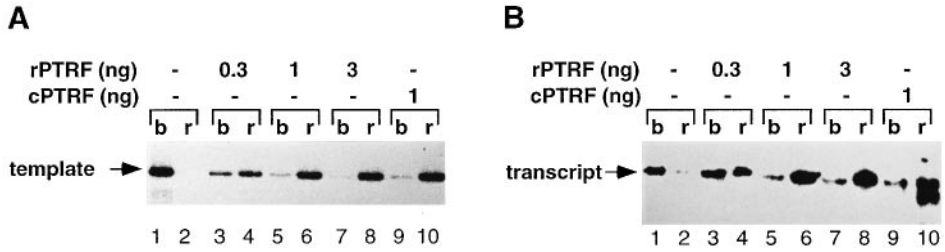

Fig. 6. PTRF mediates dissociation of ternary transcription complexes. (**A**) PTRF-dependent release of template DNA from ternary complexes containing immobilized Pol I. Ternary complexes were formed by pre-incubating bead-bound Pol I with labeled pCAT-T6-T1 template and cold nucleotides. After addition of increasing amounts of recombinant PTRF (lanes 3–8) or cellular PTRF (lanes 9 and 10), the distribution of bound and released template was analyzed. (**B**) PTRF-dependent release of transcripts from ternary complexes containing immobilized Pol I. Reactions were identical to those described in (A) except that the assays contained unlabeled template and [α-^{32}P]GTP.

also observed a strong interaction between both proteins (lane 6). Thus PTRF, by interacting with both Pol I and TTF-I, appears to serve a role in mediating the contact between TTF-I and the paused RNA polymerase.

Recombinant PTRF mediates release of both nascent transcripts and RNA polymerase I

To prove that the cloned cDNA encodes functionally active PTRF, we tested the recombinant protein in the transcript release assay. For this, PTRF was expressed in *E.coli*, purified by chromatography on Ni^{2+}-NTA–agarose and S–Sepharose, and assayed in transcription reactions containing immobilized tailed template, Pol I and TTF-I. Clearly, the majority of transcripts were released from the template in the presence of PTRF (Figure 5A, lanes 1–6), whereas no transcript release was observed in reactions containing an unrelated protein (GCN5) which was expressed and purified in parallel.

A qualitatively similar result was obtained if RNA release was not assayed with release-deficient Pol I, but on isolated paused ternary transcription complexes. Paused complexes were formed by pre-incubating Pol I with the immobilized template, NTPs, Pol I and TTF-I. The remarkable stability of paused transcription complexes allows them to be washed and thus to be depleted of excess Pol I and TTF-I. In the experiment shown in Figure 5B, bead-bound ternary complexes were isolated, washed and then incubated with increasing amounts of recombinant PTRF. Again, in the absence of PTRF, all transcripts remained associated with the template (Figure 5B, lanes 1 and 2) whereas, after addition of PTRF, the transcripts were released into the supernatant (Figure 5B, lanes 3–6). Moreover, consistent with previous results demonstrating that transcript release is an energy-independent process (Mason *et al.*, 1997a), recombinant PTRF promoted dissociation of paused ternary complexes both in the absence of NTPs and in the presence of non-hydrolyzable nucleotides (data not shown).

If the recombinant protein exerts the same functional properties as cellular PTRF, then it should facilitate release of not only transcripts but also Pol I from paused elongation complexes. To address this issue, a modified transcription assay containing immobilized Pol I was used. In the experiment shown in Figure 6A, the transcription reactions contained a labeled DNA template, TTF-I, nucleotides and Pol I that was bound to magnetic beads via antibodies against RPA116, the second largest subunit of murine Pol I (Seither and Grummt, 1996). Pol I fixed to magnetic beads is capable of supporting specific transcription (Seither *et al.*, 1998). To monitor dissociation of ternary complexes, transcription was performed with bead-bound Pol I, labeled template, TTF-I and cold nucleotides. Transcription complexes were isolated by magnetic attraction, washed, resuspended in transcription buffer and incubated in the absence and presence of PTRF. Finally, the assays were separated into bead-bound and supernatant fraction, and the distribution of labeled DNA was analyzed. In the absence of PTRF, the template was in the bead-bound fraction, indicating that it was contained within the ternary transcription complex (Figure 6A, lanes 1 and 2). However, in the presence of both recombinant PTRF (Figure 6A, lanes 3–8) and partially purified cellular PTRF (Figure 6A, lanes 9 and 10), the majority of labeled DNA was found in the soluble fraction. This result indicates

that PTRF induced dissociation of ternary transcription complexes and therefore liberated the template from bead-bound Pol I.

In parallel reactions, transcript release was measured under the same conditions, except that in these assays the template was not labeled and the reactions were supplemented with [α-^{32}P]GTP. As shown in Figure 6B, in the presence of PTRF, liberation of transcripts into the supernatant was observed, demonstrating that PTRF promotes transcript release irrespective of whether the template or the polymerase were fixed to magnetic beads. This tight correlation between PTRF-dependent release of both template DNA and nascent transcripts from immobilized ternary Pol I complexes demonstrates that PTRF is capable of dissociating stalled ternary complexes, thereby liberating both Pol I and RNA.

PTRF binds specifically to the 3′ end of pre-rRNA

As shown above, cellular PTRF binds to the 3′ end of pre-rRNA, and this binding appears to be required for transcript release. To establish whether recombinant PTRF has the same specificity with respect to binding to the U-rich element, we first compared the wild-type (pCAT-T6-T1) and the mutant template (pCAT-G6-T1) in transcript release assays using histidine-tagged PTRF expressed in *E.coli*. Consistent with the requirement for the U stretch in PTRF function, recombinant PTRF mediates transcript release from the wild-type (Figure 7A, lanes 1–6) but not from the mutant template (Figure 7A, lanes 7–12). Furthermore, like the cellular factor (Figure 1), recombinant PTRF binds to an RNA probe harboring 3′-terminal pre-rRNA sequences (Figure 7B, lanes 1–3), and substitution of the U stretch by G residues strongly impairs binding (Figure 7B, lanes 4–6). This result underscores the importance of the U run at the 3′ end of pre-rRNA for dissociation of TTF-I-stalled ternary transcription complexes and demonstrates that the recombinant protein exhibits the same functional properties as the cellular factor.

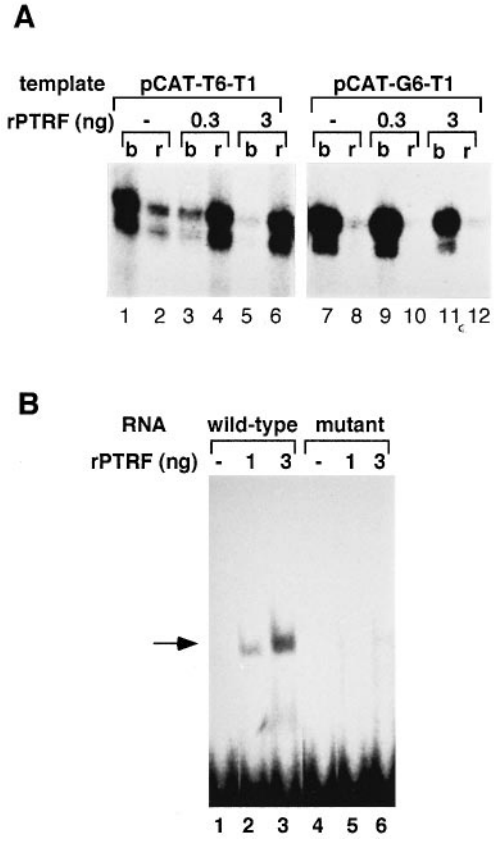

Fig. 7. Recombinant PTRF requires the T stretch upstream of the T_1 terminator. (**A**) Recombinant PTRF releases transcripts from wild-type but not mutant templates. Transcriptions were performed on bead-bound pCAT-T6-T1 (lanes 1–6) and pCAT-G6-T1 (lanes 7–12) in the absence or presence of recombinant histidine-tagged PTRF as indicated, and fractionated into template-bound (b) and released (r) transcripts. (**B**) Recombinant PTRF recognizes the nucleotide sequence of the 3′ end of pre-rRNA. Histidine-tagged PTRF was incubated with labeled RNA representing either the wild-type (lanes 1–3) or mutant version (lanes 4–6) of the 3′ end of pre-rRNA. The reactions were resolved on a 5% polyacrylamide gel and visualized by autoradiography.

Discussion

Transcription termination by Pol I is a multistep process involving pausing of the elongating polymerase, release of both the newly synthesized RNA and Pol I, and 3′-end processing of the primary transcript (Reeder and Lang, 1994, 1997; Mason *et al.*, 1998). Despite great differences in the sequences of the terminator elements and the DNA-binding proteins from species as diverse as mouse, frog and yeast, the mechanism of termination in all eukaryotes is probably very similar. All characterized Pol I terminator elements are recognized by a specific DNA-binding protein that either directly or indirectly contacts the elongating RNA polymerase and mediates the termination reaction. With the availability of cloned terminator proteins, it has been possible to establish cell-free transcription systems which terminate Pol I at the same sites as utilized *in vivo* and thus allow the study of the mechanism of transcription termination. These studies revealed that Pol I transcription termination can be separated into two mechanistically distinguishable steps. First, Pol I is paused by a DNA-bound protein, e.g. in the mouse by the transcription termination factor TTF-I bound to the 'Sal box' terminator element. Secondly, another cellular factor, termed PTRF, is required for dissociation of the paused ternary transcription complex leading to transcript release and liberation of the polymerase from the template (Mason *et al.*, 1997a). PTRF activity requires specific T-rich DNA sequences upstream of the Sal box terminator. Thus, both processes of Pol I termination, pausing and release, use different *cis*-acting elements and *trans*-acting factors.

Based on its interaction with TTF-I, we have cloned the cDNA encoding PTRF using TTF-I as bait in the yeast two-hybrid system. The sequence of PTRF is highly conserved among human, mouse and chicken. Although a search of the yeast genome database revealed no ORF with substantial homology to PTRF, there is experimental evidence that a homolog of PTRF also exists in yeast. We have demonstrated previously that murine PTRF can liberate transcripts from yeast Pol I that has been paused by Reb1p, the functional equivalent of TTF-I in yeast (Mason *et al.*, 1997b). In addition, it was reported that a factor from *Saccharomyces cerevisiae* induces dissociation of yeast Pol I when paused by the Lac repressor (Tschochner and Milkereit, 1997). Finally, two forms of

Pol I could be separated chromatographically from both mouse and yeast cells, one that is competent for transcript release on its own and one that is release-deficient and requires a cellular protein fraction to facilitate dissociation of ternary complexes (Mason *et al*., 1997a; Tschochner and Milkereit, 1997). The murine factor causes dissociation of ternary complexes arrested by Reb1p (Mason *et al*., 1997b), indicating that the surfaces of protein–protein interactions involved in Pol I transcription termination are conserved.

The finding that release-deficient Pol I preparations can be complemented by adding fractions containing PTRF activity suggests that this factor is either an accessory protein that co-purifies with but is separable from Pol I or, alternatively, a subunit of Pol I that has been dislodged during purification. The latter possibility can be excluded because the sequence of PTRF is distinct from those of the five murine Pol I subunits that have been cloned so far. Consistent with PTRF being a novel Pol I-associated factor rather than a genuine Pol I subunit, the electrophoretic mobility of PTRF is distinct from that of any known subunit of Pol I, and PTRF is not recognized by antibodies against Pol I (data not shown). Moreover, mild washing of ternary transcription complexes abolishes transcript release activity. Finally, pull-down and co-immunoprecipitation experiments revealed that PTRF interacts with both TTF-I and Pol I, a finding which suggests that PTRF may form a bridge between Pol I and TTF-I. In this scenario, PTRF traveling with the elongating polymerase would contact DNA-bound TTF-I, which in turn may induce complex dissociation. In support of this idea, we have found that PTRF, but not TTF-I, is associated with the recently described >2000 kDa murine 'Pol I holoenzyme' complex (Seither *et al.*, 1998).

Besides this network of specific protein–protein interactions, PTRF binds to RNA, and specific interaction with the 3′-terminus of pre-rRNA appears to be crucial for PTRF function. Previous work in mouse and yeast demonstrated that mutations in sequences upstream of the TTF-I- or Reb1p-binding site affect termination efficiency, suggesting that upstream elements are a universal feature of Pol I terminators. Block mutagenesis of the mouse or yeast Pol I terminator demonstrated that the upstream element constitutes an essential part of the terminator and that the U-rich sequence within the last 10–12 nucleotides of the primary transcript is required for dissociation of the Pol I ternary complex (Kuhn *et al*., 1988; Kuhn and Grummt, 1989; Lang *et al*., 1994; Reeder and Lang, 1994; Lang and Reeder, 1995). We now have demonstrated that both cellular and recombinant PTRF bind to the 3′ end of pre-rRNA, and that the U-rich sequence element is required for specific binding. Replacement of the six uridine residues by guanosines strongly impairs RNA binding. We are still ignorant as to whether or not PTRF binds to the 3′-terminus of pre-rRNA only, or whether PTRF or a functionally homologous protein may play other roles in cellular RNA metabolism, too. The fact that PTRF is a relatively abundant protein lends support to this attractive hypothesis.

In many respects, the functional properties of PTRF resemble those of the La protein. La is an autoimmune antigen that is transiently associated with the precursors of Pol III transcripts via their common 3′-terminal UUU_{OH} motif (Gottlieb and Steitz, 1989; Maraia *et al.*, 1994). The specificity for this motif reflects La's role as a transcription termination factor that mediates nascent transcript release and facilitates recycling of Pol III onto stable pre-initiation complexes. The functional similarity between La protein and PTRF is intriguing. First, like PTRF, binding of La to this 3′-terminal sequence motif mediates nascent transcript release (Maraia *et al.*, 1994). Secondly, La and PTRF transiently associate with the nascent transcripts, a feature that presumably accounts for the high levels of the respective proteins in the nucleus. Moreover, both proteins fractionate into transcriptionally inactive and active forms which, in the case of La, has been attributed to reversible phosphorylation (Fan *et al*., 1997). By analogy, our fractionation scheme used for purification of Pol I and transcription initiation factors consistently revealed a significant amount of cellular PTRF that did not co-fractionate with transcript release activity (data not shown). Whether or not inactivation of PTRF activity is due to phosphorylation or association with other cellular proteins is not yet known. Finally, perhaps the most interesting analogy between La and PTRF is their ability to stimulate transcription. Addition of either cellular or recombinant PTRF not only augments the efficiency of transcript release, but also increases the overall rate of transcription in a concentration-dependent manner (Figure 5A). While such stimulation of Pol III transcription is consistent with a role for La in increasing the efficiency of reinitiation (Maraia, 1996), this remains to be determined for PTRF.

By most criteria, recombinant PTRF is functionally equivalent to the cellular release factor characterized previously (Mason *et al*., 1997a). A comparison of the activity of cellular versus recombinant PTRF revealed functional identity in the (i) specificity of RNA binding, (ii) transcript release and (iii) stimulation of overall transcription. In contrast to factor 2, a Pol II transcript release factor from *Drosophila* (Xie and Price, 1996), neither cellular nor recombinant PTRF required ATP hydrolysis for complex dissociation as revealed by identical efficiency of transcript release in the absence and presence of nucleotides, or in the presence of the non-hydrolyzable nucleotide analogs AMP-PNP and GMP-PNP.

One difference between cellular and recombinant PTRF was noted in Pol I-associated transcript cleavage, a common feature of prokaryotic and eukaryotic transcription. 3′ end maturation of pre-rRNA and of many pre-tRNAs is accomplished by nucleolytic removal of 3′ trailer sequences. In earlier studies, we identified an exonuclease activity present in the Pol I transcription termination complex which removes a few nucleotides from the 3′ end of the nascent transcript *in vivo* and *in vitro*. The longer transcript is a precursor for processing to the shorter one (Kuhn and Grummt, 1989), and the processing activity is absent in TTF-I, but is present in release-competent Pol I (Kuhn *et al.*, 1990). It remains to be investigated whether PTRF itself possesses this cleavage activity or, by analogy to elongation factor SII which also interacts with both the 3′ end of nascent RNA and Pol II and activates the nuclease function of a Pol II elongation complex (Reines and Mote, 1993; Powell *et al.*, 1996), PTRF can induce a latent ribonuclease activity in template-

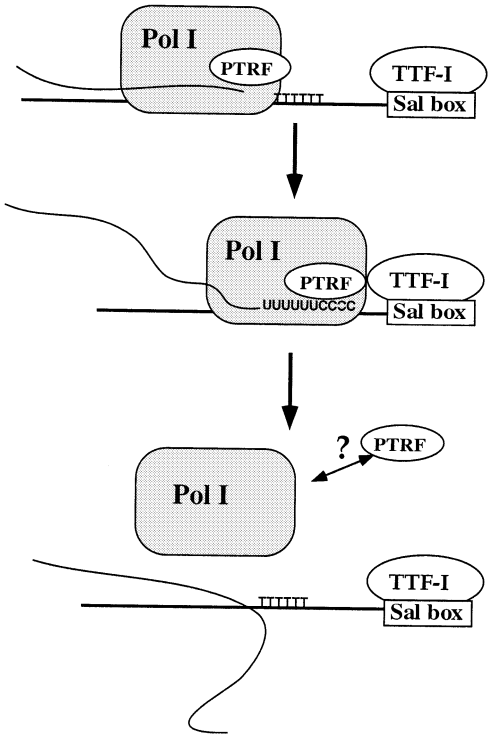

Fig. 8. Model for murine Pol I transcription termination. Pol I, together with PTRF, approaches the terminator and pauses 11 nucleotides upstream of TTF-I bound to the Sal box element. The binding of PTRF to the U-rich element in the nascent transcript is required for dissociation of the ternary complex, resulting in the release of both RNA and Pol I from the template.

engaged elongation complexes which removes a few 3′-terminal nucleotides from the terminated transcript. Alternatively, PTRF could mediate a limited backtracking of Pol I. As reported by Nudler *et al*. (1997), T-rich sequences induce *E.coli* RNA polymerase to backtrack, i.e. its active center moves backwards on the DNA and RNA leaving non-base-paired RNA at the 3′ end of the transcript. However, in contrast to the 'backsliding' reaction of *E.coli* RNA polymerase, mouse Pol I stops transcription not at the end of the U run, but at the end of the C stretch. Based on the properties of TTF-I and PTRF, we propose the following model for Pol I transcription termination (Figure 8). The approaching Pol I pauses upstream of TTF-I bound to the terminator element. It is conceivable that the collision of Pol I and TTF-I leads to a retreat of the active site of Pol I by four nucleotides, which in turn would poise the active site near the upstream U run. In this scenario, PTRF, being associated with the elongating Pol I, would be properly positioned to bind its target site on RNA. An as yet unidentified RNase activity, which is either associated with or recruited by PTRF, then removes 3′ trailer sequences to yield mature pre-rRNA ends. Although some features of this model have yet to be established, by testing such models a better understanding of the mechanism of transcription termination is within our reach.

Materials and methods

Plasmids

pCAT-T6-T1 is similar to pCAT554–650 which has been described previously (Kuhn *et al*., 1990). It contains a 151 bp fragment from the bacterial *CAT* gene (nucleotides 4853–5003 in pSV2–CAT) which is fused to a 49 bp fragment from the 3′-terminal spacer region of mouse rDNA (from +556 to +604 relative to the 3′ end of the 28S rRNA coding region). In pCAT-G6-T1, the six thymidine residues (from +566 to +571) were substituted by guanosines (Kuhn *et al*., 1988). The plasmids pBS-T6-Sma and pBS-G6-Sma were made by inserting a *Sma*I site by PCR at position +575 of the 3′-terminal fragment using pCAT-T6-T1 or pCAT-G6-T1 as a template, and the resulting PCR fragment was cloned into pBluescript II SK. pEG202TTF, pEG202TTFΔN323 and pEG202TTFΔN445 were obtained by cloning the respective derivatives of murine TTF-I (Evers *et al*., 1995) into pEG202. The cDNA corresponding to the ORF of murine PTRF was amplified by PCR using the forward primer 5′-GGAATTCCATATGGAGGATGTCACGCTCC-ATATC-3′ and the backward primer 5′-GGAATTCCTCAGTCCCTGT-CGCTCTTGTCCACCAG-3′. After digestion with *Eco*RI, the DNA fragment was cloned into the vector pJG4-5 to yield the plasmid pJG4-5PTRF. pRSETB–PTRF and pGEX–PTRF were obtained by cloning the *Eco*RI fragment derived from pJG4-5PTRF into pRSETB and pGEX-1N, respectively. The plasmids pRSET–TTF and pRSET–TTFΔ185 were described elsewhere (Evers *et al*., 1995).

Yeast two-hybrid screening

The yeast two-hybrid screening was performed essentially as described (Gyuris *et al*., 1993). The yeast strain EGY48 was co-transformed with the *lexAop–LacZ* reporter plasmid pSH18–34 together with the vector pEG202–TTF which expresses a fusion of LexA and mouse TTF-I. The resulting chimeric protein was used as a bait to screen for cDNAs that encode TTF-I-interacting proteins. A selection strain harboring the LexA–TTF-I bait was transformed with a human (Wi-38) cDNA library cloned into pJG4-5 expressing proteins fused to the B42 transcriptional activation domain (Gyuris *et al*., 1993). By screening 2×10^7 yeast transformants, 21 interacting clones were obtained; nine of them proved to be true positives containing five different cDNAs. The yeast strain EGY48, all basic plasmids and the cDNA library for the two-hybrid system were generously provided by Dr R.Brent.

Cloning of cDNA encoding murine PTRF

The sequence information of the partial human cDNA encoding PTRF (DDBJ/EMBL/GenBank accession No. AF000421) and its chicken homolog (DDBJ/EMBL/GenBank accession No. D26315) was used to clone the full-length mouse cDNA by a PCR-based approach (Frohman *et al*., 1988). Briefly, poly(A)$^+$ RNA from NIH 3T3 cells was transcribed into single-stranded cDNA with avian myeloblastosis virus (AMV) reverse transcriptase (Boehringer Mannheim) using a wobbled nested primer (5′-TCTGCG/CCGG/TGACTCCTCAATAAC/TC-3′) based on the cDNA sequence of human and chicken PTRF. The cDNA was purified, a poly(dC) tail was added with terminal transferase (Promega), and the DNA was amplified using a forward (dG)14 primer and a wobbled nested primer 5′-C/TC/ATCCATCTCC/TG/TG/CTGC/TCG/TCTCCTC-3′. The 3′-terminal part of the cDNA was cloned by 3′ RACE using an 18mer of oligo(dT) and a PTRF-specific primer 5′-TGATCTACCAGGATGAAGTCAAGC-3′. The amplified fragments of the 5′ and 3′ RACE were cloned into pBluescript II SK, sequenced and fused in-frame by ligation of overlapping fragments to yield a 1416 bp cDNA which encodes full-length (392 amino acids) murine PTRF. The sequence data have been submitted to the DDBJ/EMBL/GenBank databases under the accession number AF036249.

Transcription on immobilized tailed templates

The tailed templates were prepared as described (Kuhn *et al*., 1990; Mason *et al*., 1997a). Briefly, the plasmids were cut by *Bgl*II and a 14 nucleotide oligonucleotide 3′-ACCAAAAAAACTAG-5′ was ligated to the cohesive ends to create a 10 nucleotide 3′ overhang. The template was cut with *Hin*dIII and the free oligonucleotides were removed by precipitating the DNA with 7.5% polyethylene glycol 6000 in the presence of 0.9 M NaCl. For immobilization, biotin-14-dATP was incorporated into the *Hin*dIII restriction site using Klenow enzyme. The biotinylated template (10 μg) was bound to 500 μl of streptavidin magnetic beads (Dynal) and incubated with bovine serum albumin (BSA) and phosphatidylcholine (5 mg/ml each) to block non-specific binding sites as described (Mason *et al*., 1997a).

Transcription on tailed templates was performed as described (Mason *et al*., 1997a) unless otherwise indicated. The 25 μl reactions containing 5 μl (100 ng) of bead-bound template, 12 mM Tris–HCl (pH 7.9), 5 mM $MgCl_2$, 0.06 mM EDTA, 12% glycerol, 70 mM KCl and 0.5 mM UpG dinucleotide (Sigma) were pre-incubated for 10 min at 30°C with 0.1–0.5 U of Pol I and 30 ng of murine TTF-I. Transcription was started by

the addition of 600 mM each of ATP, UTP and CTP, 12.5 mM GTP and 8 μCi of [α-^{32}P]GTP. After incubation for 10 min in the presence or absence of PTRF, transcripts were separated into template-bound and released fractions. Transcription was stopped by addition of an equal volume of stop buffer (0.2 M ammonium acetate pH 5.2, 0.4% SDS, 1 mg/ml yeast tRNA). The RNA was extracted, precipitated with ethanol and resolved on 6% polyacrylamide/7 M urea gels.

Transcription with immobilized RNA polymerase I

An aliquot (2 μg) of affinity-purified polyclonal antibodies against RPA116, the second largest subunit of Pol I, was bound to 25 μl of magnetic beads (Dynal) as described (Seither and Grummt, 1996; Seither *et al*., 1997). Before use, the beads were equilibrated in buffer AM-100 (20 mM Tris–HCl pH 7.9, 5 mM $MgCl_2$, 100 mM KCl, 0.1 mM EDTA, 20% glycerol) supplemented with 2 mg/ml BSA, insulin and phosphatidylcholine to block non-specific interactions. The packed α-RPA116 beads were incubated with 25 μl (5 U) of purified Pol I (MonoQ fraction) for 2 h at 4°C in 100 μl of buffer AM-150 in the presence of 0.1% NP-40. The beads were washed with 100 μl of the same buffer and twice with AM-100. Ten μl of bead-bound Pol I (corresponding to 3 μl of the MonoQ Pol I fraction) were used in 25 μl transcription reactions containing 200 ng of tailed pCAT-T6-T1 and 30 ng of TTF-I. After pre-incubation for 10 min at 30°C, NTPs were added and transcription was carried out for 10 min. Then ternary complexes were isolated by magnetic attraction, washed and incubated further for 10 min in the presence or absence of PTRF. The reactions were fractionated into bead-bound and supernatant fraction, and nucleic acids were extracted and resolved on a 6% polyacrylamide/7 M urea gel. When the release of the template from ternary complexes was measured, the reactions were supplemented with 0.5 ng of labeled template DNA (3000 c.p.m.).

Purification of RNA polymerase I, TTF-I and cellular PTRF

Mouse Pol I was purified by chromatography on DEAE–Sepharose, heparin–Ultrogel, S–Sepharose and MonoQ HR 10/10 as described (Schnapp and Grummt, 1996). The peak Pol I fraction from the MonoQ column was used to separate release-deficient from release-competent Pol I (Mason *et al.*, 1997a). For this, the fractions eluting at 320 mM KCl were purified by gel filtration on Superdex 200 (HiLoad) 26/60 run in buffer AM-120 (20 mM HEPES–KOH pH 7.9, 120 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol, 10% glycerol). Pol I-containing fractions were applied immediately onto MonoQ HR 5/5 ($MonoQ_{II}$) and eluted with a 15 ml linear gradient from 200–450 mM KCl. The catalytic activity of Pol I was determined in a non-specific transcription assay. One unit of activity is defined as the amount of RNA polymerase that incorporates 1 pmol of [^{3}H]UMP into acid-precipitable material within 30 min at 30°C in an assay containing 7.5 μg of calf thymus DNA as template (Schnapp and Grummt, 1996). Release-deficient Pol I fractions were identified in transcript release assays using bead-bound tailed templates. Murine TTF-I was expressed by infecting Sf9 cells with recombinant baculovirus encoding histidine-tagged TTFΔN185, an N-terminally truncated mutant of TTF-I which is expressed efficiently and exhibits higher DNA-binding and termination activity than full-length recombinant TTF-I (Sander *et al.*, 1996). Purification on Ni^{2+}-NTA–agarose has been described (Sander *et al*., 1996). PTRF was partially purified from mouse nuclear or cytoplasmic extracts as described (Mason *et al*., 1997a). The amount of PTRF in cellular fractions was estimated on immunoblots using anti-PTRF antibodies.

RNA binding experiments

An 80 nucleotide labeled RNA probe corresponding to the natural end of mouse pre-rRNA (from +554 to +575 relative to the 3′ end of 28S rRNA) was synthesized by T7 RNA polymerase (Promega) from *Sma*I-digested template pBS-T6-Sma or pBS-G6-Sma. The RNAs were purified and 3000 c.p.m. of the ^{32}P-labeled RNA were incubated in 20 μl reactions containing 20 mM Tris–HCl (pH 7.9), 70 mM KCl, 5 mM $MgCl_2$, 25 μg/ml of tRNA, 0.5 μg/ml of BSA and varying amounts of cellular or recombinant PTRF. The reactions were incubated on ice for 30 min and subjected to electrophoresis on 5% polyacrylamide gels in 0.5× TBE buffer. RNA–protein complexes were visualized by autoradiography.

In vitro interaction assays

Yeast extract containing an HA-tagged fusion of PTRFΔN150 with the B42 transcription activation domain was prepared as described (Formosa *et al*., 1991). Fifteen μg of extract protein were incubated with 10 μl of histidine-tagged TTFΔN185 bound to Ni^{2+}-NTA beads (Qiagen) or control Ni^{2+}-NTA beads, respectively. Beads were washed with 10 vols of buffer AM-100 (20 mM HEPES–KOH pH 7.9; 100 mM KCl; 5 mM $MgCl_2$; 0.2 mM EDTA; 1 mM dithiothreitol; 20% glycerol) and eluted with 1 M KCl. The fractions were separated on 10% SDS–polyacrylamide gels and analyzed on immunoblots using anti-HA antibodies.

GST–PTRF or GST were expressed in *E.coli* BL21(DE3)pLysS and purified by adsorption onto glutathione–Sepharose (Pharmacia). Glutathione–Sepharose beads bearing either GST alone or the GST–PTRF fusions were blocked with 2 mg/ml BSA and insulin and incubated with ^{35}S-labeled recombinant mTTF-I which was generated in a coupled *in vitro* transcription/translation rabbit reticulocyte lysate system (Promega). Then 10 μl of the translation reaction were diluted with 20 μl of buffer AM-100/0.05% NP-40 and applied onto a microcolumn containing 10 μl of packed beads carrying either GST or GST–PTRF. The columns were washed with 10 volumes of loading buffer and bound proteins were eluted with 3 volumes of buffer AM-2000/0.05% NP-40. Proteins were separated by SDS–PAGE and visualized by autoradiography.

Immunoprecipitations and immunoblots

Polyclonal antibodies against recombinant PTRF and the third largest subunit of Pol I (α-PAF/RPA53) were purified by coupling 2 mg of the respective antigen to a 0.3 ml of Affi-Gel 10 column (BioRad) according to the manufacturer's instructions. Five μg of IgGs were coupled to 25 μl of magnetic beads covered with sheep anti-rabbit immunoglobulins (Dynal) according to the manufacturer's instructions. Before use, the magnetic beads were equilibrated in buffer AM-100 supplemented with 2 mg/ml BSA, insulin and phosphatidylcholine. The packed beads were incubated with 50 μl of a fractionated nuclear extract (DEAE-280 fraction) for 4 h at 4°C in 100 μl of buffer AM-100 in the presence of 0.1% NP-40. The beads were washed sequentially in 100 μl of buffer AM-100/0.5% NP-40, AM-200/0.1% NP-40 and twice in AM-100/0.1% NP-40. For Western blot analysis, proteins were eluted off the magnetic beads by boiling in 20 μl of sample buffer. After electrophoresis, the polypeptides were transferred to nitrocellulose membranes and probed with either affinity-purified chicken anti-PTRF or anti-RPA116 antibodies (Seither and Grummt, 1996).

Acknowledgements

We thank Andreas Hunziker and Hajo Delius for DNA sequencing, Werner Zwerschke for help with the yeast two-hybrid system and Roger Brent for the yeast strain EGY48, all basic plasmids and the cDNA library used in the two-hybrid screen. This work was supported by the Deutsche Forschungsgemeinschaft (GHGP) and the Fond der Chemischen Industrie.

References

Bartsch,I., Schoneberg,C. and Grummt,I. (1987) Evolutionary changes of sequences and factors that direct transcription termination of human and mouse ribosomal genes. *Mol. Cell. Biol.*, **7**, 2521–2529.

Evers,R., Smid,A., Rudloff,U., Lottspeich,F. and Grummt,I. (1995) Different domains of the murine RNA polymerase I-specific termination factor mTTF-I serve distinct functions in transcription termination. *EMBO J.*, **14**, 1248–1256.

Fan,H., Sakulich,A.L., Goodier,J.L., Zhang,X., Qin,J. and Maraia,R.J. (1997) Phosphorylation of the human La antigen on serine 366 can regulate recycling of RNA polymerase III transcription complexes. *Cell*, **88**, 707–715.

Fields,S. and Song,O. (1989) A novel genetic system to detect protein–protein interactions. *Nature*, **340**, 245–246.

Formosa,T., Barry,J., Alberts,B.M. and Greenblatt,J. (1991) Using protein affinity chromatography to probe structure of protein machines. *Methods Enzymol.*, **208**, 24–45.

Frohman,M.A., Dush,M.K. and Martin,G.R. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl Acad. Sci. USA*, **85**, 8998–9002.

Gottlieb,E. and Steitz,J.A. (1989) Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J.*, **8**, 851–861.

Grummt,I., Maier,U., Öhrlein,A., Hassouna,N. and Bachellerie,J.P. (1985) Transcription of mouse rDNA terminates downstream of the 3′ end of 28S RNA and involves interaction of factors with repeated sequences in the 3′ spacer. *Cell*, **43**, 801–810.

Grummt,I., Rosenbauer,H., Niedermeyer,I., Maier,U. and Öhrlein,A. (1986) A repeated 18 bp sequence motif in the mouse rDNA spacer mediates binding of a nuclear factor and transcription termination. *Cell*, **45**, 837–846.

Gyuris,J., Golemis,E., Chertkov,H. and Brent,R. (1993) Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell*, **75**, 791–803.

Kuhn,A. and Grummt,I. (1989) 3′ End formation of mouse pre-rRNA involves both transcription termination and a specific processing reaction. *Genes Dev.*, **3**, 224–231.

Kuhn,A., Normann,A., Bartsch,I. and Grummt,I. (1988) The mouse ribosomal gene terminator consists of three functionally separable sequence elements. *EMBO J.*, **7**, 1497–1502.

Kuhn,A., Bartsch,I. and Grummt,I. (1990) Specific interaction of the murine transcription termination factor TTF I with class-I RNA polymerases *Nature*, **344**, 559–562.

Lang,W.H. and Reeder,R.H. (1995) Transcription termination of RNA polymerase I due to a T-rich element interacting with Reb1p. *Proc. Natl Acad. Sci. USA*, **92**, 9781–9785.

Lang,W.H., Morrow,B.E., Ju,Q., Warner,J.R. and Reeder,R.H. (1994) A model for transcription termination by RNA polymerase I. *Cell*, **79**, 527–534.

Manley,J.L. and Proudfoot,N.J. (1994) RNA 3′ ends: formation and function—meeting review. *Genes Dev.*, **8**, 259–264.

Maraia,R.J. (1996) Transcription termination factor La is also an initiation factor for RNA polymerase III. *Proc. Natl Acad. Sci. USA*, **93**, 3383–3387.

Maraia,R.J., Kenan,D.J. and Keene,J.D. (1994) Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. *Mol. Cell. Biol.*, **14**, 2147–2158.

Mason,S.W., Sander,E.E. and Grummt,I. (1997a) Identification of a transcript release activity acting on ternary transcription complexes containing murine RNA polymerase I. *EMBO J.*, **16**, 163–172.

Mason,S.W., Wallisch,M. and Grummt,I. (1997b) RNA polymerase I transcription termination: similar mechanisms are employed by yeast and mammals. *J. Mol. Biol.*, **268**, 229–234.

Mason,S.W., Sander,E.E., Evers,R. and Grummt,I. (1998) Termination of mammalian RNA polymerase I transcription. In Paule,M.R. (ed.), *Transcription of Eukaryotic Ribosomal RNA Genes by RNA Polymerase I*. R.G.Landes Company, Austin, TX, pp. 179–194.

Nudler,E., Mustaev,A., Lukhtanov,E. and Goldfarb,A. (1997) The RNA–DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. *Cell*, **89**, 33–41.

Powell,W., Bartholomew,B. and Reines,D. (1996) Elongation factor SII contacts the 3′ end of RNA in the RNA polymerase II elongation complex. *J. Biol. Chem.*, **271**, 22301–22304.

Reeder,R.H. and Lang,W. (1994) The mechanism of transcription termination by RNA polymerase I. *Mol. Microbiol.*, **12**, 11–15.

Reeder,R.H. and Lang,W.H. (1997) Terminating transcription in eukaryotes: lessons learned from RNA polymerase I. *Trends Biochem. Sci.*, **22**, 473–477.

Reines,D. and Mote,J. (1993) Elongation factor SII-dependent transcription by RNA polymerase II through a sequence-specific DNA-binding protein. *Proc. Natl Acad. Sci. USA*, **90**, 1917–1921.

Sander,E.E., Mason,S.W., Munz,C. and Grummt,I. (1996) The amino-terminal domain of the transcription termination factor TTF-I causes protein oligomerization and inhibition of DNA binding. *Nucleic Acids Res.*, **24**, 3677–3684.

Sawada,K., Agata,K. and Eguchi,G. (1996) Characterization of terminally differentiated cell state by categorizing cDNA clones derived from chicken lens fibers. *Int. J. Dev. Biol.*, **40**, 531–535.

Schnapp,A. and Grummt,I. (1996) Purification, assay and properties of RNA polymerase I and class I-specific transcription factors in mouse. *Methods Enzymol.*, **273**, 233–248.

Seither,P. and Grummt,I. (1996) Molecular cloning of RPA2, gene encoding the second largest subunit of mouse RNA polymerase I. *Genomics*, **37**, 135–139.

Seither,P., Zatsepina,P. and Hoffmann,M., Grummt,I. (1997) Constitutive and strong association of PAF53 with RNA polymerase I. *Chromosoma*, **106**, 216–225.

Seither,P., Iben,S. and Grummt,I. (1998) A subpopulation of mammalian RNA polymerase exists as a holoenzyme with associated basal factors required for transcription initiation. *J. Mol. Biol.*, **275**, 43–53.

Senger,M., Glatting,K.H., Ritter,O. and Suhai,S. (1995) X-HUSAR, an X-based graphical interphase for the analysis of genomic sequences. *Comput. Methods Programs Biomed.*, **46**, 131–141.

Shilatifard,A., Conaway,J.W. and Conaway,R.C. (1997) Mechanism and regulation of transcriptional elongation and termination by RNA polymerase II. *Curr. Opin. Genet. Dev.*, **7**, 199–204.

Tschochner,H. and Milkereit,P. (1997) RNA polymerase I from *S.cerevisiae* depends on an additional factor to release terminated transcripts from the template. *FEBS Lett.*, **410**, 461–466.

Xie,Z. and Price,D.H. (1996) Purification of an RNA polymerase II transcript release factor from *Drosophila*. *J. Biol. Chem.*, **271**, 11043–11046.

Received January 27, 1998; revised and accepted March 13, 1998