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Oligomerization of the transcription termination factor TTF-I: implications for the structural organization of ribosomal transcription units

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ABSTRACT

Mammalian ribosomal genes are flanked at their 5′ and 3′ ends by terminator sequences which are recognized by the transcription termination factor TTF-I. The occurrence of the same binding site upstream and downstream of the gene raises the possibility that TTF-I can interact with both sequences simultaneously and thus brings the terminator in the vicinity of the gene promoter by looping out the pre-rRNA coding sequence. To test this model, we have examined the ability of TTF-I to oligomerize and found that both full-length and N-terminally truncated versions of TTF-I form stable oligomeric structures. At least two domains of TTF-I located within the 184 N-terminal and 445 C-terminal amino acids, respectively, mediate the self-association of several TTF-I molecules. In support of the looping model, TTF-I is capable of linking two separate DNA fragments via binding to the target sites. This result indicates that in addition to its function in transcription termination, TTF-I may serve a role in the structural organization of the ribosomal genes which may be important for maintaining the high loading density of RNA polymerase I on active rRNA genes.

INTRODUCTION

In eukaryotes, transcription of the genes that code for ribosomal RNA (rDNA) accounts for up to 80% of cellular RNA which is being synthesized at any instant in a rapidly growing cell. This high transcriptional activity is brought about by maximal density of RNA polymerase I (Pol I) together with an amplification of the number of transcription units. In mammals, ~200 copies of rDNA per haploid genome are required to synthesize the more than a million ribosomes per generation that are needed to maintain the translational capacity of the new daughter cells. In most species, rDNA is arranged in tandem head-to-tail repeats in which a transcribed region alternates with an intergenic spacer region. Consistent with the role of rRNAs in ribosome structure and function, the gene regions that code for 18S, 5.8S and 28S rRNAs are highly conserved. The intergenic spacer, on the other hand, exhibits a pronounced heterogeneity, both in length and sequence. However, even though the sequence of regulatory elements that govern Pol I initiation and termination vary considerably between species (for reviews see 1,2), the overall structural organization of the rDNA repeats is similar. Several types of regulatory elements are located in the intergenic spacer, including (i) the gene promoter at the 5′ end of the pre-rRNA coding region, (ii) a transcription terminator immediately upstream of the gene promoter, (iii) enhancer elements that stimulate transcription, (iv) one or more spacer promoters, and (v) terminator elements at the 3′ end of the pre-rRNA coding region. Specific transcription factors bind directly or via protein–protein interactions to these regions and thus promote the synthesis of faithfully initiated and terminated pre-rRNA.

Eukaryotic ribosomal transcription units are flanked both at the 5′ and 3′ side by one or more terminator elements. In mouse, the 18 bp terminator motif, termed ‘Sal box’ because it contains a restriction site for *Sal*I, is repeated several times (T_1–T_8) downstream of the 3′ end of the pre-rRNA coding region and has been shown to mediate transcription termination *in vivo* and *in vitro* (3–5). Alterations in the ‘Sal box’ that reduce binding of the interacting factor TTF-I (for **T**ranscription **T**ermination **F**actor) also impair transcription termination. There is marked sequence divergence between terminator elements from different organisms, such as mammals, frog and yeast. The molecular mechanism of Pol I transcription termination, however, is probably similar or even identical in these diverse species. All characterized Pol I terminators function in only one orientation and bind a termination factor which presumably contacts the elongating RNA polymerase (for review, see 6). The cDNAs for murine and human TTF-I have been cloned and deletion analysis has revealed functionally distinct domains of the protein (7,8). Interestingly, the DNA binding activity of recombinant TTF-I (TTF-I_{p130}) has been found to be masked in the intact protein (9). Removal of the N-terminal part of TTF-I, on the other hand, greatly augments DNA binding. These findings suggested that the N-terminus of TTF-I may inhibit DNA binding via intermolecular protein–protein interactions. Consistent with this idea, we found that the N-terminal 184 amino acids of TTF-I can form stable oligomers in solution and repress DNA binding when fused to a heterologous DNA binding domain (10).

The fact that binding sites for the termination protein are present both upstream and downstream of the rDNA transcription

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unit suggests a functional linkage between transcription termination and initiation. A model has been proposed in which each rDNA transcription unit forms a loop which juxtaposes the promoter and the terminator element (11,12). Thus, Pol I molecules having terminated at the downstream terminator could be transferred directly from the 3′ end of the gene to the promoter of the adjacent rDNA unit without entering the free pool. The finding that a sequence motif that is almost identical to the downstream terminator elements is also located adjacent to the rDNA promoter suggests that simultaneous binding of a sequence-specific protein to both the upstream and downstream terminators may connect the 5′ and 3′ end of the rDNA and therefore mediate DNA looping. In the loop structures that are supposed to be formed, interaction between the upstream and downstream terminators of the same or adjacent transcription units can be juxtaposed. Planta and collaborators have suggested that REB1p, with indentical binding sites near the promoter and the 3′ end of the rRNA operon, is causally involved in loop formation (12). The experimental data presented in this study strongly support this model. We demonstrate that TTF-I, the murine homologue of yeast REB1p can self-associate and form oligomeric structures both in solution and when bound to DNA. The intermolecular interactions between different TTF-I molecules, in turn, enable the factor to interact simultaneously with two separate DNA fragments bearing a TTF-I binding site. The results are compatible with the hypothesis that TTF-I may link the proximal and distal part of the rDNA transcription units as distinct loop structures.

MATERIALS AND METHODS

Plasmid constructs

Expression vectors containing histidine-tagged TTF-I_{p130}, TTF-ΔN185, TTFΔN323 and TTFΔN445 in pRSET (Invitrogen) were described by Evers *et al.* (7). For expression in baculovirus-infected Sf9 cells, *Nde*I–*Hin*dIII fragments containing the histidine-tag and TTF-I sequences from the pRSET constructs were cloned into pBacPAK9 (Clontech). GST-TTFΔN323 was generated by cloning a *Bam*HI (blunt)/*Eco*RI fragment from pRSET-TTFΔN323 into pGEX-3X (Pharmacia).

Expression and purification of TTF-I from baculovirus-infected insect cells

Proteins were expressed by infecting 2.5×10^8 Sf9 cells with recombinant baculovirus. The cells were harvested after 48 h, rinsed in PBS, resuspended in 3 vol of lysis buffer (50 mM HEPES–KOH, pH 7.8; 300 mM KCl; 5 mM $MgCl_2$; 1 mM PMSF; 1 μg/ml leupeptine), and were lysed by sonification followed by addition of 0.5% NP-40 and centrifugation. The supernatant was incubated with NTA–agarose beads (Quiagen) for 30 min at 4°C in the presence of 1 mM imidazole. The beads were washed with 20 column volumes of buffer 1 (50 mM HEPES–KOH, pH 7.8; 300 mM KCl; 5 mM $MgCl_2$; 0.5% NP-40; 1 mM imidazole; 1 mM PMSF; 1 μg/ml leupeptine), 20 vol of buffer 2 (same as buffer 1 with 1 M KCl) and 20 vol of buffer 3 (same as buffer 1 with 10 mM imidazole). Proteins were eluted with 20 mM HEPES–KOH, pH 7.8; 100 mM KCl; 5 mM $MgCl_2$; 200 mM imidazole; 1 mM PMSF; 1 μg/ml leupeptine and dialysed against buffer AM-100 (20 mM Tris–HCl, pH 7.9; 5 mM $MgCl_2$; 100 mM KCl; 0.1 mM EDTA; 20% glycerol; 2 mM DTE).

Gel filtration of TTF-I

To determine the size of native TTF-I by gel filtration, 25–50 μl aliquots of ^{35}S-labeled TTF-I derivatives synthesized by *in vitro* translation (Promega) were centrifuged and passed over a Superdex200 FPLC (HR10/30, Pharmacia) column at a flow rate of 0.5 ml/min in buffer AM-100 without glycerol (20 mM Tris–HCl, pH 7.9; 5 mM $MgCl_2$; 100 mM KCl; 0.1 mM EDTA; 2 mM DTE). The fractions were precipitated with trichloroacetic acid, analyzed by SDS–PAGE and autoradiography, and the amount of TTF-I in individual fractions was quantified with a PhosphorImager.

Protein–protein interaction assays

TTFΔN323 fused to glutathione S-transferase (GST-TTFΔN323) was expressed in *Escherichia coli* BL21(DE3) and purified on glutathione–Sepharose beads as specified by the manufacturer (Pharmacia). 50 μl assays contained 2 μg of fusion protein bound to 10 μl of glutathione–agarose beads and 2–5 μl of ^{35}S-labeled full-length or mutant forms of TTF-I in buffer AM-100. The reactions were incubated for 30 min at room temperature and washed three times with buffer AM-100. The washed beads were boiled in sample buffer and the released proteins were electrophoresed on 10% SDS–polyacrylamide gels.

DNA binding assays

20 μl reactions containing TTF-I and 5 fmoles of a ^{32}P-labeled 246 bp PCR fragment covering rDNA sequences from –232 to +14 (relative to the transcription start site) were incubated for 15 min on ice in binding buffer (12 mM Tris–HCl, pH 8.0; 85 mM KCl; 5 mM $MgCl_2$; 0.1 mM EDTA; 1 mM DTE; 8% glycerol; 2 ng/μl BSA; 4 ng/μl phage λ DNA cut with *Hae*III; 0.1% NP-40), and protein–DNA complexes were separated by electrophoresis on 4% polyacrylamide gels in 0.5× TBE buffer (50 mM Tris-borate, pH 8.3; 1.3 mM EDTA) at 4°C and 10 mA. For competition, a double-stranded 'Sal box' oligonucleotide (SB; upper strand 5′-GATCCTTCGGAGGTCGACCAGTACTCCGGGCGACA-3′) or mutant oligonucleotide (SB*, 5′-GATCCTTCGGAGCGCGACCAGTACTCCGGGCGACA-3′) was used (9).

To assay TTF-I binding to two different DNA fragments, a 160 bp PCR fragment containing the terminator element T_2 (9) was generated using a biotinylated primer. The fragment was attached to streptavidin-coated magnetic beads (Dynal) according to the manufacturers specifications. Ten fmoles of immobilized DNA, 10 fmoles (40 000 c.p.m.) of ^{32}P-labeled 'Sal box' oligonucleotide (SB or SB*) and TTF-I were incubated for 30 min at 30°C in binding buffer containing 0.5% NP-40. Protein–DNA complexes were isolated in a magnetic field, washed in 50 μl buffer AM-100, eluted with 15 μl of loading buffer (10 mM Tris–HCl, pH 8.0; 5 mM EDTA; 1% SDS; 30% glycerol; 0.01% bromphenolblue; 0.01% xylene cyanol) and analyzed on a 12% native polyacrylamide gel.

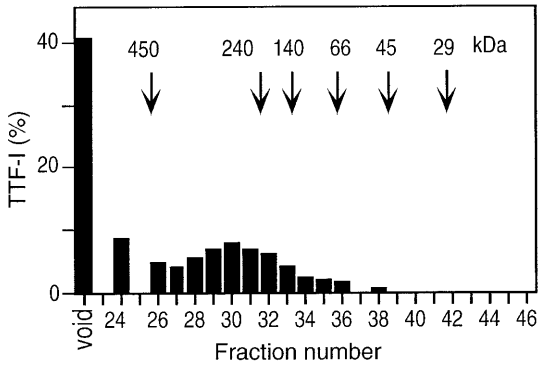

Figure 1. Identification of oligomeric states of TTF-I in the absence of DNA. ^{35}S-labeled TTFΔN445 was chromatographed on a Superdex200 gel filtration column. The graph shows the amount of TTFΔN445 present in each fraction. The calculated molecular mass of monomeric TTFΔN445 is 43 kDa. The positions of molecular mass standards (*E.coli* RNA polymerase, 450 kDa; catalase, 240 kDa; lactate dehydrogenase, 140 kDa; bovine serum albumin, 66 kDa; albumin, 45 kDa; carbonic anhydrase, 29 kDa) are marked by arrows.

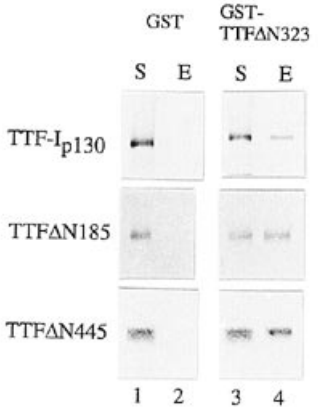

Figure 2. Interaction between different TTF-I molecules. Glutathione–agarose beads bearing GST alone (lanes 1 and 2) or GST-TTFΔN323 (lanes 3 and 4) were tested for binding of ^{35}S-labeled TTF-I dervatives. 10% of the supernatant fractions (S; lanes 1 and 3) and the eluate of the bound fraction (E; lanes 2 and 4) are presented to allow an assessment of binding efficiency.

RESULTS

TTF-I forms oligomers in solution

In a recent study we have compared DNA binding and termination activity of recombinant full-length TTF-I (TTF-I$_{p130}$) with two deletion mutants lacking 184 and 322 N-terminal amino acids (TTFΔN185 and TTFΔN323). These studies revealed that the DNA binding of TTF-I$_{p130}$ is at least one order of magnitude lower than that of the deletion mutants indicating that the N-terminus represses the interaction of TTF-I with DNA (10). Moreover, on glycerol gradients both full-length TTF as well as a polypeptide encompassing the N-terminal 320 amino acids (TTF1–320) sedimented much faster than expected for a monomeric protein. These and other data suggested that the N-terminal domain of TTF-I has the potential to oligomerize with itself and that oligomerization of TTF-I may influence its DNA binding activity.

To investigate whether the ability of TTF-I to form stable oligomers in solution was mediated exclusively by the N-terminus or whether the C-terminal part of TTF-I is also able to interact with itself, we determined the native size of TTFΔN445, a mutant harboring the C-terminal DNA binding domain. For this, radiolabeled protein, synthesized by *in vitro* translation, was subjected to gel filtration on a Superdex200 column. Figure 1 shows the distribution of TTFΔN445 in individual column fractions as analyzed by SDS–PAGE and autoradiography. Similar to previous studies showing that TTF-I$_{p130}$ forms oligomers in solution (10), a significant part of the N-terminally truncated mutant TTFΔN445 also eluted in the void volume or in fractions which represent molecular sizes larger than expected for a monomeric 43 kDa protein. Thus, not only the very N-terminus, but also the C-terminal part of TTF-I can mediate self-association of several TTF-I molecules. The same result was obtained with highly purified TTF-I derivatives that were expressed in baculovirus-infected Sf9 cells and purified by affinity chromatography (data not shown) indicating that the intermolecular interaction of several TTF-I molecules is due to oligomerization of TTF-I and is not dependent on other factors present in the translation lysates.

To substantiate these findings, protein–protein binding or 'pull-down' assays were performed. For this, TTFΔN323, a deletion mutant which encodes amino acids 323–833 of TTF-I was fused in frame to the C-terminus of glutathione-S-transferase. This fusion protein, GST-TTFΔN323, as well as GST alone, was expressed in *E.coli*, purified on glutathione–agarose, and equal amounts of the immobilized proteins were incubated with ^{35}S-methionine-labeled TTF-I derivatives. As seen in Figure 2, GST-TTFΔN323 (lane 4), but not GST alone (lane 2), was able to retain significant amounts of full-length TTF-I$_{p130}$ (~10% of input), suggesting that homomeric interactions occur between two or more TTF-I molecules. Significantly, the two N-terminally truncated mutants TTFΔN185 and TTFΔN445 bound to GST-TTFΔN323 with similar efficiency and specificity as the full-length protein. This result demonstrates that not only the N-terminal 184 amino acids (10), but also the C-terminal half of TTF-I (amino acids 445–833) mediates intermolecular interactions between two ore more TTF-I molecules.

Oligomerization of TTF-I bound to DNA

Next we investigated whether TTF-I would also form multimeric complexes when bound to DNA. In the experiment shown in Figure 3, increasing amounts of TTFΔN185 purified from baculovirus-infected insect cells were incubated with a labeled rDNA fragment containing one TTF-I binding site and the resulting protein–DNA complexes were analyzed by electrophoresis. In the presence of 20 fmoles of TTF-I, the DNA probe was quantitatively converted into a distinct DNA–TTF-I complex (complex C1, lane 2) which exhibits a lower electrophoretic mobility than free DNA. Significantly, when the amount of TTF-I was increased to 500 fmoles, complex C1 was converted into a more slowly migrating complex (C2, lane 3). This finding, together with the observation that TTF-I forms oligomers in solution, suggests that complex C2 most likely contains several TTF-I molecules. If this assumption was correct, then addition of a 'Sal box' oligonucleotide which competes for TTF-I binding should convert complex C2 into complex C1. The competitions shown in Figure 3 (lanes 4–7) support this view. At saturating amounts of TTF-I (20 fmoles), an excess of 'Sal box' oligonucleotide

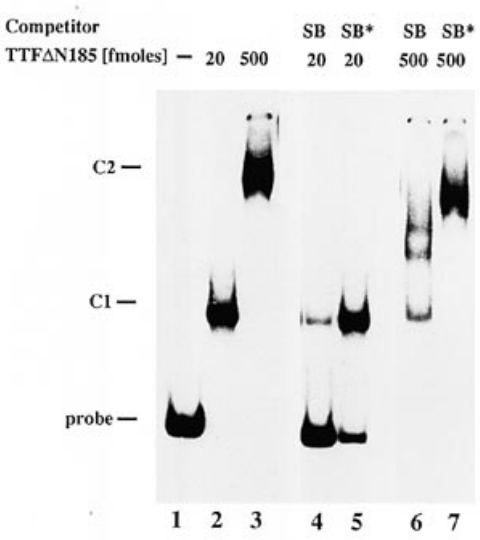

Figure 3. Multiple TTF-I molecules bind to a 3′ terminal rDNA fragment containing the promoter-proximal terminator T_0. 20 or 500 fmoles of purified TTFΔN185 were incubated with 5 fmol of the rDNA fragment and resulting protein–DNA complexes (designated as C1 and C2) were analyzed on a 4% native polyacrylamide gel (lanes 1–3). Competition reactions were carried out with with 250 fmol of wild-type (SB) or mutant (SB*) 'Sal box' oligonucleotide (lanes 4–7).

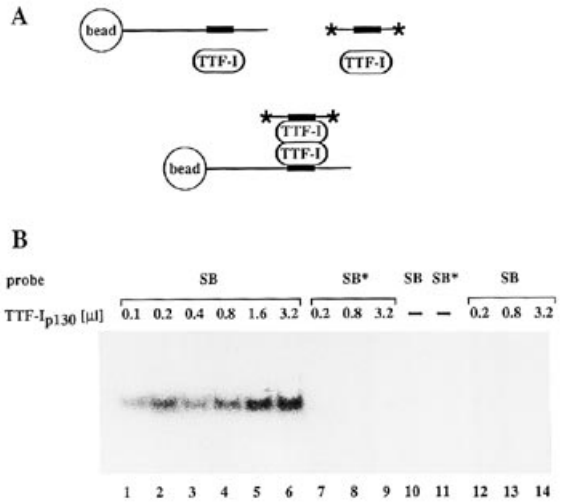

Figure 4. Multimers of TTF-I can link separate DNA molecules. (**A**) Schematic illustration of the magnetic bead assay. For details, refer to the text. (**B**) Increasing amounts of recombinant TTF-I_{p130} were incubated with labeled 'Sal box' oligonucleotide (SB, lanes 1–6) or mutant oligonucleotide (SB*; lanes 7–9) and a terminator-containing DNA fragment that was immobilized on magnetic beads. Bead-bound protein–DNA complexes were washed in buffer AM-100, separated on a 12% polyacrylamide gel and visualized by autoradiography. Reactions in lanes 10 and 11 did not contain TTF-I. In lanes 12–14 increasing amounts of TTF-I_{p130} were incubated with labeled SB oligonucleotide, non-immobilized DNA fragment and DNA-free magnetic beads to monitor non-specific binding.

containing the TTF-I target sequence (SB), but not a mutant oligonucleotide (SB*), efficiently competed for TTF-I binding to the labeled DNA probe (lanes 4 and 5). If the competitions were performed at high concentrations (500 fmoles) of TTF-I, addition of wild-type 'Sal box' oligonucleotide, but not the mutant, prevented formation of complex C2. Consistent with a stepwise dissociation of the complex C2, complex C1 and distinct intermediates were observed (lanes 6 and 7). No free probe was generated, because of the high amounts of TTF-I in the assay required to produce complex C2. Significantly, the same complexes migrating between complex C1 and C2 were observed if intermediate TTF-I concentrations, i.e. between 20 and 500 fmoles, were used (data not shown). This result suggests that at high molar ratios of TTF-I to DNA several TTF-I molecules bind simultaneously to the 'Sal box' target sequence. Although these data do not allow definite conclusions about the stoichiometry of TTF-I binding, the observation that complex C2 is more prominent than the two intermediate complexes indicates that complex C2 is a multimeric complex, presumably a tetramer, which is more stable than dimers or trimers.

TTF-I can link two separate DNA fragments

The ability of TTF-I to form multimers when bound to DNA raises the possibility that TTF-I could connect two DNA segments containing TTF-I binding sites. This possibility is particular intriguing because it would be compatible with the 'ribomotor' model which proposes that each rDNA transcription unit forms a loop which places the gene promoter and terminator into close proximity (11). Our data suggest that TTF-I may be the *trans*-acting factor that bridges the 5′ and the 3′ end of the transcription unit by binding simultaneously to the upstream and the downstream terminator(s). If this model is correct, then TTF-I should be able to link spatially separated DNA fragments. To test this idea, a 'bridging assay' was designed, as illustrated in Figure 4A. In this assay, a DNA fragment containing a TTF-I binding site was immobilized on magnetic beads and incubated with a radiolabeled 'Sal box' oligonucleotide in the absence and presence of TTF-I_{p130}. Protein–DNA complexes formed on the immobilized DNA were isolated by magnetic separation and analyzed for the presence of labeled oligonucleotide. As shown in Figure 4B, association of labeled 'Sal box' oligonucleotide with bead-bound DNA was dependent on TTF-I. Increasing the input of TTF-I_{p130} increased the amount of labeled oligonucleotide (SB) in the bead-bound fraction (lanes 1–6). This interaction was dependent on TTF-I binding to its target sequence, because a labeled mutant oligonucleotide (SB*) that is not recognized by TTF-I did not associate with the immobilized DNA (lanes 7–9). This result indicates that DNA-bound TTF-I can link two separate DNA segments. In control reactions that lack TTF-I (lanes 10 and 11) or contain a non-immobilized DNA fragment (lanes 12–14) no significant levels of radiolabeled 'Sal box' oligonucleotide were found to be associated with the beads. Together, this finding demonstrates that TTF-I may tether different DNA molecules. However, the overall amount of 'sandwich' complexes containing two 'Sal box'-containing DNA fragments, hold together by TTF-I, is low. Whether this is due to experimental manipulation or due to the instability of this kind of complexes is not known.

Central regions of TTF-I are required to link separate DNA fragments

To delineate the region of TTF-I which tethers separate DNA molecules, various TTF-I mutants were tested for their ability to physically link the immobilized rDNA fragment and the radiolabeled 'Sal box' oligonucleotide. Purified baculovirus-expressed

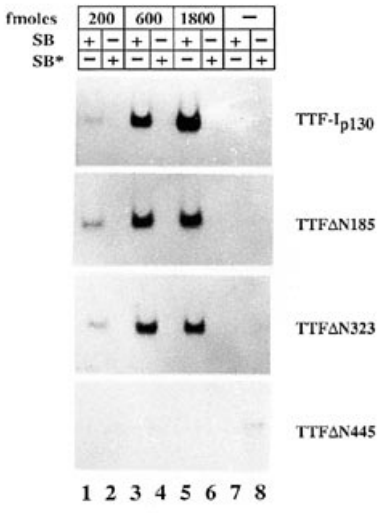

Figure 5. Amino acids 323–445 from TTF-I are required for tethering separate DNA fragments. Binding assays with an immobilized DNA fragment and labeled wild-type 'Sal box' oligonucleotide (SB; lanes 1, 3, 5 and 7) or mutant oligonucleotide (SB*; lanes 2, 4, 6 and 8) were performed in the absence of TTF-I (lanes 7 and 8) or in the presence of increasing amounts of baculovirus-expressed TTF-I$_{p130}$, TTFΔN185, TTFΔN323 and TTFΔN445 as described in Figure 4.

full-length TTF-I (TTF-I$_{p130}$) as well as the deletion mutants TTFΔN185, TTFΔN323 and TTFΔN445 were analyzed in the 'bridging assay' described above (Fig. 5). Significantly, TTFΔN185 and TTFΔN323 were as active as intact TTF-I in tethering the two DNA fragments, indicating that deletion of 322 N-terminal amino acids did not affect the simultaneous interaction of TTF-I with spatially separated DNA fragments. In contrast, mutant TTFΔN445 was inactive in this assay (lanes 1, 3 and 5). This is an interesting observation, because sequences between amino acids 323 and 445 have been shown to play an important role in both transcription termination (7) and in TTF-I directed chromatin remodeling (13). Thus, the failure of TTFΔN445 to link physically separated DNA fragments supports the view that the C-terminus on its own, including the DNA binding function of TTF-I and the ability to oligomerize, is not sufficient for TTF-I function, but requires sequences between amino acids 323 and 445.

DISCUSSION

In order to complete the transcription cycle, RNA polymerase must undergo termination which includes cessation of elongation and the release of both the terminated RNA chains and RNA polymerase from the template. The common view of reinitiation is that RNA polymerase needs to be released in order to be recruited by preinitiation complexes to start a new transcription cycle. However, the finding that in yeast the rDNA terminator maps within a DNA region that enhances transcription initiation suggested a functional linkage between termination and initiation. A model has been proposed which implies that each rDNA transcription unit forms a loop which may channel polymerases directly to the promoter after termination, thus bypassing the pool of free Pol I molecules (11). This is an attractive model because it reveals a possible mechanism by which the high level of rDNA transcription is accomplished. A looping mechanism would maintain a high loading density of the rDNA transcription unit by ensuring efficient recycling of Pol I from the 3′ tail to the 5′ head of active genes. In support of this model, micrographs of chromatin spreads from *Bombyx mori* and *Drosophila* tissue culture cells show active rDNA transcription units as loops separated by intergenic spacers (14).

In this study we provide experimental support for this model. Our data suggest that TTF-I, besides its function in termination of Pol I transcription, serves a role in the structural organization of active rDNA transcription units. The following lines of evidence argue that interaction between several TTF-I molecules bound to either the promoter proximal or distal terminator elements may connect the 5′ and 3′ end of the gene. First, the position of the upstream transcription terminator T_0 with respect to essential promoter elements has been conserved. In mouse, rat, human, *Xenopus laevis* and *X.borealis*, T_0 is located ~200 bp upstream of the transcription initiation site and therefore, may be part of the promoter itself. T_0 has been shown to stimulate transcription initiation *in vivo* to some extent (15–17). This positive effect on transcription has been interpreted to be the result of shielding the promoter from polymerases that read through from spacer promoters, thereby inactivating or 'occluding' productive initiation complexes (16,18). However, consistent with the looping model, the upstream terminator also stimulates transcription initiation by a mechanism which is dependent on the helical alignment between the terminator and the rDNA promoter (19).

A second argument for TTF-I connecting distant rDNA regions is the observation that TTF-I forms oligomeric structures. We demonstrate that the ability of TTF-I to associate with itself is not restricted to the N-terminal part, but that an additional oligomerization domain is also contained within the C-terminal region including amino acids 445–833. Interestingly, the two oligomerization domains appear to be functionally different. The N-terminal domain (which resides between amino acids 1 and 184) has previously been shown to form stable oligomers in solution and to repress the DNA binding activity of full-length TTF-I (10). TTFΔN445, the mutant that specifically binds DNA but is inactive in transcription termination (7), also forms oligomeric complexes in solution. However, this mutant fails to link two physically separated DNA molecules. Apparently, oligomerization of TTF-I in solution *per se* is not sufficient for linking separate DNA segments. This result underscores the importance of the central part of TTF-I including amino acids from 323 to 445 in functions other than DNA binding. We propose that this central part of TTF-I, together with the C-terminal DNA binding domain, is not only essential for transcription termination (7) and chromatin remodeling (13), but also plays a crucial structural role in organizing the rDNA transcription units and spacer regions.

Stable protein–protein mediated DNA loops may provide a general mechanism by which distant DNA sites modulate gene expression. Multimeric structures, such as homo-multimers of E2 dimers and of Sp1 tetramers, frequently assemble at loop junctures (20–23). One example of how homomeric protein oligomerization may affect gene expression via a DNA looping mechanism is the tumor suppressor protein p53. Natural p53 binding sites placed adjacent to TATA elements effectively stimulate transcription by p53 (24). p53 exists as tetramers and multiples of tetramers in solution (25–27). In a model promoter containing multiple copies of the consensus sequence, p53 has

been found to assemble oligomeric complexes by a novel mechanism which stacks tetramer on top of tetramer. Morover, stacked oligomers link separated binding sites via DNA loops and promote transcriptional enhancement *in vivo* (28). By analogy to TTF-I, p53 can assemble oligomers by two distinct domains, a C-terminal tetramerization domain and a non-tetrameric oligomerization domain that loops separated consensus sites by protein–protein interactions.

The possibility that polymerase 'hand-over' from the end of one gene to another rDNA promoter may be responsible for the high polymerase density seen on ribosomal genes has previously been tested in microinjected oocytes (29) as well as in cultured kidney cells (30). The studies revealed that high rates of transcription initiation do not depend upon polymerase passing from one repeat to the next. We have also performed a series of *in vitro* transcription experiments to find out whether the presence of both an upstream and a downstream terminator would increase the initiation frequency on artificial ribosomal minigenes. We consistently observed transcriptional enhancement by TTF-I (data not shown). However, this enhancement was not due to communication between the promoter-proximal terminator and the downstream TTF-I binding site, but was brought about by the downstream terminator alone. These and other experiments indicate that TTF-I stimulates transcription, presumably by facilitating the reinitiation reaction**.** Therefore, a final proof whether or not RNA polymerase I is 'handed over' from the downstream terminator to the gene promoter is still lacking. Nevertheless, our data suggest that TTF-I may be causally involved in maintaining a loop structure of the rDNA transcription units. Whether the interaction between the upstream and downstream terminators is mediated exclusively by TTF-I, or whether it involves additional proteins that may anchor the rDNA to the nucle(ol)ar matrix in a highly ordered, linear fashion remains to be investigated.

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