SV40 large T antigen binds to the TBP–TAF₁ complex SL1 and coactivates ribosomal RNA transcription

Weiguo Zhai, JoAnn Tuan, and Lucio Comai

Department of Molecular Microbiology and Immunology, University of Southern California, School of Medicine, Los Angeles, California 90033 USA

SV40 large T antigen is a multifunctional regulatory protein that plays a key role in the viral life cycle and can stimulate cell proliferation. To accomplish this, large T antigen has to control the expression of cellular genes involved in cell cycle progression and cell growth. rRNA synthesis by RNA polymerase I (Pol I) is tightly associated with cell growth and proliferation, and previous studies indicated that large T antigen up-regulates RNA Pol I transcription in SV40-infected cells. How this process occurs is currently unclear. To investigate the mechanisms of large T antigen stimulation of RNA Pol I transcription, we have established an in vitro transcription system that is responsive to large T antigen. Here, we show that recombinant large T antigen stimulates Pol I transcription reconstituted with purified RNA Pol I, UBF, and the TBP/TAF complex SL1. Immunoprecipitation experiments revealed that large T antigen directly binds to SL1, in vitro, as well as in SV40-infected cells. In addition, our data indicate that this interaction occurs by direct association with three SL1 subunits, namely TBP, TAF₄₈, and TAF₁₁₀. Transcription studies with large T antigen deletion mutants show that the 538-amino-acid amino-terminal domain is necessary for full stimulation of Pol I transcription. Importantly, mutants that no longer bind to SL1 are also unable to stimulate Pol I transcription. This indicates that recruitment of large T antigen to the rRNA promoter by SL1 constitutes a crucial step in the activation process. Taken together with recent studies on large T antigen activation of RNA Pol II transcription, these results suggest that viral modulation of genes involved in cell proliferation involves direct targeting of promoter-specific TBP/TAF complexes (i.e., SL1 or TFIID) by large T antigen.

[Key Words: Transcription; SL1; TAFs; Pol I; large T antigen]

Received January 24, 1997; revised version accepted May 6, 1997.

RNA polymerase I (Pol I) directs RNA synthesis from a single class of genes, the rRNA genes, which are found in multiple tandem arrayed copies in the nucleoli of eukaryotic cells [Reeder 1994; Jacob 1995; Moss and Stefanovsky 1995]. rRNA transcription plays a critical role in ribosome biogenesis, and changes in the transcription rate of RNA Pol I are associated with profound alterations in the growth rate of the cell [Sollner-Webb and Tower 1986; Sommerville 1985]. In humans, transcription by RNA Pol I requires at least two transcription factors in addition to RNA Pol I: the upstream binding factor [UBF], and the selectivity factor SL1 [Learned et al. 1985, 1986]. Biochemical studies have established that a complex set of interactions of UBF and SL1 with each other and with the DNA template are necessary to form an active complex and to initiate promoter-specific transcription [Learned et al. 1986; Bell et al. 1988]. In vitro studies indicated that UBF, a high mobility group (HMG) box sequence-specific DNA-binding protein, binds to the rDNA promoter and recruits the selectivity factor SL1 [Bell et al. 1988; Jantzen et al. 1990, 1992]. SL1 is required to direct initiation from the rRNA promoter and plays a crucial role in the promoter recognition properties of the rRNA transcriptional apparatus [Learned et al. 1985; Clos et al. 1986]. Human SL1 is a species-specific factor, and when added to a mouse cell-free transcription extract, it has the ability to reprogram that extract to recognize a human rRNA promoter [Mishima et al. 1982; Learned et al. 1985; Bell et al. 1990, Schnapp et al. 1991]. SL1 is analogous to the TFIID and TFIIIB factors in the Pol II and Pol III transcription systems and consists of TATA-binding protein [TBP], plus three distinct TBP-associated factors [TAFs] essential to reconstitute Pol I transcription [Comai et al. 1992; Eberhart et al. 1993; Hernandez 1993]. In the presence of UBF, the SL1 complex binds the DNA template and extends the DNase I footprint to include the species-specific element [SSE] [Bell et al. 1988; Safrany et al. 1989].

In response to physiological and environmental changes, cells must regulate the expression level of metabolically important genes such as the rRNA genes.
Extracellular stimuli, as well as many pathological phenomena, including amino acids starvation, toxic lesion, aging, cancer, and viral infections, dramatically modulate RNA Pol I transcription [Grummt et al. 1976; Mishima and Muramatsu 1979; Cavanaugh and Thompson 1986; Hammond and Bowman 1988; Reeder 1994; Jacob 1995]. Early studies showed that infection of mammalian cells with SV40 induced rRNA synthesis [May et al. 1976; Pockl and Wintersburger 1980]. Further analysis indicated that the product of the viral gene A, the large tumor antigen (large T antigen), was directly involved in this process [Ide et al. 1977; Soprano et al. 1981; Learned et al. 1983]. Large T antigen is a multifunctional protein that has been shown to induce cell proliferation, transformation, and tumor formation in animals [for review, see Fanning 1992; Conzen and Cole 1994]. Several studies have indicated that large T antigen can regulate cell growth, in part, by binding and inactivating the tumor suppressor gene products pRB and p53 [Hinds and Weinberg 1994; Neovins 1994]. In addition, large T antigen can transactivate cellular genes, and several studies have indicated that this process is mediated by specific protein–protein interactions with multiple components of the transcriptional machinery (Gruda et al. 1993; Berger et al. 1996; Damania and Alwine 1996; Johnston et al. 1996]. These alterations in the transcriptional activity of specific cellular genes most likely play an important role in the growth-promoting activity induced by large T antigen.

Transcription of the rRNA genes is necessary for cell growth and proliferation, therefore, it is not surprising that the stimulation of Pol I transcription is one of the strategies adopted by large T antigen to induce and sustain cell growth. Experiments with isolated nuclei and cell-free extracts suggested that the stimulation of rRNA synthesis occurred primarily at the transcriptional level [Ide et al. 1977; Learned et al. 1983]. In addition, in isolated nuclei, stimulation of rRNA synthesis was abolished by pretreatment with T antigen antibodies, but not with preimmune serum [Ide et al. 1977]. Although these studies established the stimulatory effect of large T antigen on Pol I transcription, the mechanisms by which T antigen affects rRNA synthesis, and the cellular factors involved in this phenomenon, remain unknown.

In this study we investigate the molecular mechanisms of stimulation of RNA Pol I transcription by large T antigen. First, we show that recombinant large T antigen can stimulate transcription from the rRNA promoter in transfection assays and in a cell-free system with purified RNA Pol I, UBF, and SL1. Second, we provide evidence that large T antigen binds to the SL1 complex through interactions with TAFI48, TAFI10, and TBP. Finally, we show that the region of large T antigen that binds to the TAFi and TBP is also necessary for stimulation of transcription by RNA Pol I. In summary, our findings suggest that large T antigen is recruited to the rRNA promoter by binding to the TBP/TAFi complex (i.e., SL1), and this protein–protein interaction plays a crucial role in activation of transcription.

Figure 1. Transactivation of a ribosomal gene construct by cotransfection with a large T antigen-expressing vector. Transfection were performed as described in Material and Methods, and Pol I-specific transcripts synthesized from the rRNA reporter gene prHu3CAT were detected by primer extension. When not indicated 3 μg (lanes 1,2) and 6 μg (lanes 3,4), of either pCMV–asT or pCMV–LT were used, respectively. The specific transcript yielded a 123-nucleotide cDNA fragment. To account for losses during the processing of the RNAs, a 5' end-labeled single-stranded DNA oligomer (60 nucleotides) was added to each reaction. The primer extension data were quantified using a PhosphorImager [Molecular Dynamics], and activity was expressed as fold induction relative to control transfection (pCMV–asT).

| pCMV–LT     | + | + | + | 3ug | 6ug | 12ug |
|-------------|---|---|---|-----|-----|------|
| pCMV–asT    | + | + | + | 1.5x | 2.6x | 4.4x |
| fold induction | 4.8x | 4.7x | | 1.5x | 2.6x | 4.4x |

Results

T antigen can stimulate rRNA synthesis in a defined RNA Pol I transcription system

Early studies indicated that large T antigen can stimulate RNA Pol I transcription. To further establish these findings we tested whether large T antigen could activate the rRNA gene in transfection assays in HeLa cells. A human rRNA promoter construct fused to the CAT gene was cotransfected into HeLa cells with a plasmid expressing either a sense [pCMV–LT (large T)] or an antisense [pCMV–asT (antisense T)] large T antigen. Figure 1 shows the primer extension analysis of the RNA isolated from the transfected cells. Transfection of the promoter construct plasmid produced a 123-nucleotide fragment, which corresponds to an RNA molecule initiated at the bona fide RNA Pol I transcription initiation site.
Deletion of the Pol I promoter region completely abolished transcription from the reporter plasmid [data not shown]. The results of this experiment indicated that at each amount of reporter tested, the addition of large T antigen-expressing plasmid resulted in approximately fourfold activation of the rRNA gene. In addition, co-transfection of increasing amounts of pCMV-LT plasmid resulted in proportionally higher stimulatory activity. Therefore, these data provide further evidence that large T antigen can activate RNA Pol I transcription in human cells.

To study in more detail the mechanism of RNA Pol I stimulation by large T antigen, we then established a cell-free transcription system that could mimic the in vivo process. As a first step, rRNA was transcribed using HeLa cell nuclear extract in the absence or presence of recombinant large T antigen purified from Sf9 insect cells. Large T antigen protein was purified to apparent homogeneity using either conventional chromatography or antibody-affinity chromatography (Clark et al. 1983; Simanis and Lane 1985). As shown in Figure 2A, addition of purified T antigen to a HeLa cell nuclear extract resulted in an increase in the level of rRNA synthesis (lanes 2,3). Quantitation of the in vitro-synthesized RNA indicates that large T antigen can stimulate RNA Pol I transcription up to three- to fourfold. Identical results were obtained using recombinant large T antigen purified by either conventional or affinity chromatography. These results established that large T antigen, when added to HeLa nuclear extract, stimulated RNA Pol I transcription to levels comparable to the ones that have been reported in vivo (this work; May et al. 1976).

We next investigated the set of factors necessary for large T antigen stimulation of rRNA transcription. For this purpose, RNA Pol I, SL1, and UBF were purified from HeLa nuclear extracts. As expected, purified SL1, in addition to Pol I and UBF, was required to reconstitute transcription, both in the absence and the presence of large T antigen [Fig. 2B, lanes 1–3]. More importantly, when purified T antigen was added to the transcription reaction, we observed a strong stimulation of rRNA synthesis, and the level of stimulation was proportional to the amount of recombinant large T antigen [lanes 4–8]. As in the previous experiment, a similar stimulatory effect on rRNA synthesis was observed with large T antigen purified by either conventional or affinity chromatography. Taken together, these results suggest that purified SL1, UBF, and RNA Pol I are necessary and sufficient to support the large T antigen-mediated stimulation of rRNA synthesis.

We also tested whether another viral transactivator protein, the adenovirus 13S-E1A protein, could also activate Pol I transcription. When increasing amounts of purified glutathione S-transferase (GST)-E1A-13S fusion protein
protein were added to either a crude nuclear extract or a reconstituted transcription system with partially purified Pol I, UBF, and SL1, we did not observe any detectable change in the level of RNA Pol I transcription (Fig. 2C,D). Therefore, we conclude that the ability to stimulate RNA Pol I transcription is an intrinsic property of SV40 large T antigen.

Large T antigen associates with the SL1 complex in SV40-infected cells

The finding that the stimulatory effect of T antigen on rRNA synthesis could be reconstituted with purified RNA Pol I, SL1, and UBF strongly suggested that this regulatory process was primarily mediated at the level of transcription. Thus, one possibility would be that large T antigen is recruited to the rRNA promoter through protein–DNA or protein–protein interactions. The ability of T antigen to interact specifically with sequences in the rRNA promoter has been tested by us and others. Footprinting and DNA-binding assays indicated that there are no specific binding sites for T antigen in the promoter region of the human rRNA gene [Learned et al. 1983; L. Comai, unpubl.]. Therefore, we postulated that large T antigen may interact with one or more of the Pol I transcription factors. To determine whether large T antigen was associated with SL1 and/or UBF in vivo, HeLa cells were infected with SV40 at a m.o.i. of 20 PFU/cell. Forty-eight hours after infection, the cells were harvested and nuclear extracts were prepared. The nuclear extracts from the infected cells and from mock-infected cells were then incubated with monoclonal antibodies raised against large T antigen, and the resulting immunocomplex was precipitated with protein A-Sepharose beads. The immunoprecipitation products were then analyzed by SDS-PAGE and subsequently immunoblotted with antibodies against TBP, TAFI63, and UBF [Fig. 3]. The results of this experiment revealed that in SV40-infected cells, large T antigen was associated with SL1, as indicated by the presence of TAFI110 [lane 3], TAFI63 [lane 6], and TBP [lane 9] in the immunoprecipitation product. On the other hand, SL1 was not immunoprecipitated from mock-infected HeLa extracts, indicating that the monoclonal antibody used in this study did not cross-react with any of the SL1 subunits. In addition, UBF did not coimmunoprecipitate with large T antigen, indicating that these two proteins did not interact with each other [lane 12]. Thus, our results indicate that large T antigen can associate with the SL1 complex in vivo and provide the first evidence that a protein–protein interaction may play a critical role in the stimulatory process.

To further establish the specificity of this protein–protein interaction, the nuclear extracts from the SV40-infected cells were fractionated by heparin–agarose chromatography. Aliquots of the flowthrough, washes, and eluted fractions were then separated on an SDS-polyacrylamide gel and the presence of large T antigen was determined by Western blot analysis. Individual fractions from the column were also assayed for SL1 activity in a reconstituted transcription assay with partially purified RNA Pol I and UBF [data not shown]. As shown in Figure 4A, large T antigen was found in the flowthrough [lane 2], which also includes the TFIID fraction [Tanese et al. 1991; Comai et al. 1992, data not shown]. In addition, a significant portion of large T antigen eluted at higher salts and copurified with the SL1 activity [lane 4].

To determine whether the subpopulation of large T antigen that eluted at high salts from the heparin–agarose was associated with the SL1 complex, this fraction was incubated with monoclonal antibodies raised against large T antigen and the resulting complex was resolved by SDS-PAGE. After transfer to nitrocellulose, the blots were probed with antibody against each of the SL1 subunits. As shown in Figure 4B, the SL1 complex [TBP and TAFs] from the SV40-infected cells [lanes 2,5,8], but not from the mock-infected cells [lanes 3,6,9], coprecipitated with large T antigen. Moreover, because DNase I was added to the extract prior to and during the immunoprecipitation step, the association of large T an-
Interactions between SV40 large T antigen and SL1 complex. Crude nuclear extracts prepared from SV40-infected HeLa cells in TM/0.4 M KCl were loaded onto a heparin-agarose column. After extensive washes with TM/0.4 M KCl buffer, the SL1 complex was eluted with TM/0.8 M KCl. The eluted fractions (lanes 4–9), one flow-through fraction (lane 2), one fraction from the wash (lane 3), and an aliquot of the original nuclear extract (lane 1) were resolved on an SDS-polyacrylamide gel and analyzed by Western blot using monoclonal antibodies against large T antigen. The SL1 activity of each fraction was assessed using in vitro transcription assays as described previously. [B] Coimmunoprecipitation of large T antigen and SL1. SL1 was purified from nuclear extracts prepared from either SV40-infected or mock-infected HeLa cells as described above. The SL1 fractions were then dialyzed to 0.1 M KCl in TM buffer and used in immunoprecipitation reactions with monoclonal antibody pAb 101 (anti-large T) cross-linked to protein A-agarose beads. The resulting immune complex was split into three aliquots, which were then resolved by SDS-PAGE and analyzed by Western blotting using antibodies against TAF110 [lanes 1–3], TAF63 [lanes 4–6], and TBP [lanes 7–9]. Arrows indicate the position of each protein. Immunoprecipitation from either SV40-infected cells [lanes 2,5,8] or mock-infected cells [lanes 3,6,9] are as indicated. An aliquot of the SL1 fraction [lanes 1,4,7] was used as a positive control in the Western blot analysis. The asterisk indicates cross-reactivity with the antibody heavy chain. (C) An SL1 fraction prepared from SV40-infected cells was incubated with either affinity-purified TBP antibodies [lane 1] or affinity-purified GST antibodies [negative control, lane 2]. The immunoprecipitation products were resolved by SDS-PAGE and analyzed by Western blotting using anti-large T antigen antibodies. Purified recombinant large T antigen was loaded in lane 3.

Interactions between SV40 large T antigen and TBP–TAFs

To better understand the association between large T antigen and SL1, we then proceeded to identify which subunit of SL1 makes direct contact with large T antigen. In vitro-translated [35S]methionine-labeled TAF48, TAF63, TAF110, and TBP were incubated with a GST–large T antigen fusion protein immobilized on affinity resin. After extensive washing, the resulting protein complexes were resolved by SDS-PAGE and analyzed by autoradiography. Input and bound radiolabeled proteins were also quantified using a PhosphorImager. As shown in Figure 5A, TAF110, TAF48, and TBP bound efficiently to large T antigen [lanes 4,12,16]. On the other hand, no significant interaction was observed with TAF63 [lane 8]. To ensure that the interactions were not mediated by nucleic acids, in a parallel experiment, ethidium bromide [200 μg/ml] was added to each binding reaction without any detectable effect on the results [data not shown]. In addition, we also showed that each of the TAFs interacted with GST–TBP [lanes 3,7,11] and that TBP bound to Flag-tagged TAF48 [lane 15], as reported previously [Comai et al. 1994]. As determined in the in vivo experiments, there was no significant interaction between large T antigen and UBF [lane 20], which, as shown previously, could bind efficiently to GST–TBP [lane 19; Beckmann et al. 1995]. The results of these protein–protein interaction assays were confirmed in coinfecction experiments in Sf9 cells using recombinant baculoviruses expressing large T antigen, TBP, UBF, and TAFs [W. Zhai and L. Comai, unpubl.].

Although the previous experiments suggested that TAF110, TAF48, and TBP were important for the association of large T antigen with SL1, we did not defini-
Zhai et al.

Figure 5. (A) Protein–protein interactions among large T antigen and TBP, TAF₆₅, and UBF. TAF₁₁₀, TAF₆₃, TAF₄₈, TBP, and UBF were in vitro translated and labeled with [³⁵S]methionine. GST (lanes 2, 6, 10, 14, 18), GST–large T antigen (lanes 4, 8, 12, 16, 20) and Flag-tagged TAF₄₈ (lane 15) were expressed in Sf9 cells using recombinant baculoviruses. GST–TBP (lanes 3, 7, 11, 15) was expressed and purified from E. coli. GST and GST–fusion proteins were immobilized on glutathione–Sepharose resin (Pharmacia). Flag-tagged TAF₄₈ was immobilized on anti-Flag M2 beads (Kodak), and subsequently incubated with the radiolabeled proteins. After several washes, the bound complexes were analyzed by SDS–PAGE and autoradiography. Ten percent of the total [³⁵S]–labeled protein used for each binding assay was loaded in the input lanes (lanes labeled i). (B) Flag-tagged TAFs, GST–large T antigen [GST–WT-T] and GST–small t antigen [GST–small-t] were purified as described in Materials and Methods. (♦) The corresponding purified protein. The identity of each of the TAFs was verified by Western blot analysis. High molecular weight bands in lanes 1 and 2 are Sf9 contaminant protein, which copurify with TAF₄₈ and TAF₆₃ on DEAE–Sepharose and anti-Flag M2 affinity gel (see Materials and Methods). Some of the lower molecular weight bands in the TAF₁₁₀ lane cross-react with the antibodies and most likely represent TAF₁₁₀ breakdown products. The purified GST–large T and GST–small t antigen were mixed with purified TAFs as described in the text, and after several hours of incubation at 4°C, the complexes were collected by precipitation with glutathione–Sepharose beads. The bound products were resolved by SDS–PAGE and transferred to nitrocellulose, and blots were probed with antibodies against the individual TAFs. Lanes i show 20% of the TAFs used in each reaction. Lanes t and T represent small t antigen and large T antigen binding reactions, respectively.

TAF₁₁₀, TAF₄₈, and TBP interact with the amino-terminal region of large T antigen

Several of the biochemical activities of large T antigen have been assigned to discrete functional domains using deletion and point mutant analysis (Fanning 1992). To identify the regions of T antigen involved in the interaction with TBP and TAFs, we tested a set of T antigen deletion mutants in a series of protein-binding assays. The large T antigen mutants used in this study, containing deletion at either the carboxyl terminus [TN1–3], the amino terminus [TC1–3], or both termini [TM1], are represented schematically in Figure 6A. The large T antigen mutants were cloned into baculovirus expression vectors and transfected into Sf9 cells for the production of recombinant viruses. Recombinant proteins were expressed as GST fusions and affinity purified using glutathione–Sepharose resin. A typical silver-stained SDS–polyacrylamide gel of the various fusion proteins eluted from GST affinity beads with 20 mM reduced glutathione is shown in Figure 6B. For the protein–protein interaction assays, TAF₄₈, TAF₆₃, TAF₁₁₀, and TBP were translated in vitro and labeled with [³⁵S]methionine. The radiolabeled proteins were then incubated with GST, GST–large T antigen [GST–WT-T], each of the GST–large T antigen mutants, and GST–small t antigen bound...
whereas binding to TAFI110 was not affected and binding relative to the protein input. As shown in Figure 7, large T antigen mutant TN1, which contains the amino-terminal region from amino acid 362 to 538, showed no significant interaction with each of the TAFs and TBP. Interestingly, small t antigen did not bind to TBP or TAFs. Because large T and small t antigen share the amino-terminal 82 amino acids, our results suggest that this region of large T is not directly involved in the interactions with TBP and the TAFs. As discussed previously, large T antigen did not bind to TAF63.

In summary, these experiments indicate that the interactions with TAF110, TAF148, and TBP are mediated by the amino terminus of large T antigen. In addition, the different affinities of TAFs and TBP to the individual deletion mutants suggest, most likely, that distinct surfaces within the amino-terminal region of large T antigen mediate the interaction with different SL1 subunits. Additional binding experiments between this set of large T antigen mutants and an integral SL1 complex confirmed that the amino-terminal 436 amino acids of large T antigen were required for efficient binding to SL1 [data not shown].

The amino-terminal portion of large T antigen is also required for stimulation of Pol I transcription

The previous protein–protein interaction studies suggested, most likely, that large T antigen was brought to the rRNA promoter via interactions with three subunits of the SL1 complex. However, the role of these interactions in the stimulatory process remained unclear. To establish the functional significance of large T antigen–SL1 interaction and to identify regions of large T antigen involved in the stimulation of rRNA synthesis, the aforementioned set of mutants was tested in the in vitro transcription assays. GST fusion proteins were purified by affinity chromatography using glutathione–Sepharose resin and used in transcription reactions with purified RNA Pol I, SL1, and UBF. As shown in Figure 8, addition of increasing amounts of wild-type large T antigen stimulates rRNA synthesis [lanes 6–8], as compared to a reaction that contained no large T antigen [lane 5] or GST alone [lanes 2–4]. Moreover, GST–TN1, which contains the first 538 amino acids of large T antigen, retained the ability to activate rRNA synthesis as efficiently as the wild-type protein [lanes 10–12]. However, further deletion of the carboxy-terminal region (TN2, TN3) caused a sharp decrease in the stimulation of RNA Pol I transcription [lanes 14–16 and 18–20]. Addition of large T antigen amino-terminal mutants (TC1, TC2, TC3), a mutant containing the internal region from amino acid 362 to 538 (TM1), or small t antigen did not cause any detectable stimulation of transcription [lanes 22–24, 26–28, 30–32, 34–36, and 38–40, respectively]. These results suggested that the amino-terminal portion of large T antigen, from amino acid 1 to 538, has an important role in the stimulation of Pol I transcription. The observation that a shorter portion of large T antigen, which contains the first 361 amino acids, did not show any Pol I stimulatory activity, suggesting that the region between amino acids 361 and 538 was necessary for this
Figure 7. Interactions of large T antigen mutants with TAF\textsubscript{110}, TAF\textsubscript{63}, TAF\textsubscript{48}, and TBP. GST, GST–large T antigen, GST–large T antigen mutants, and GST–small t antigen were expressed in Sf9 cells using recombinant baculovirus and immobilized on glutathione-affinity resin (see Fig. 6 for nomenclature). Radiolabeled TAF\textsubscript{110}, TAF\textsubscript{63}, TAF\textsubscript{48}, and TBP were incubated with the various GST fusion proteins, and after washing the beads extensively, the resulting protein complexes were resolved by SDS-PAGE. Radiolabeled bound proteins were quantified using a PhosphorImager and plotted as percentage bound (\%) protein relative to the input for each reaction. The type of bar representing each factor is shown on the side legend. Numbers next to each large T antigen mutant refer to the amino acid residues delimiting each mutant.

This regulatory process. However, because the mutant TM1 [amino acids 362–538] was transcriptionally inactive, this portion of large T antigen was clearly not sufficient to enhance Pol I activity [lanes 34–36]. Moreover, these results demonstrate further that the association of large T antigen with SL1 plays an important role in this process, as deletion mutants TC1, TC2, TC3, and TM1, which did not bind to SL1, were unable to stimulate Pol I transcription.

Figure 8. Transcriptional stimulation in vitro of RNA Pol I by large T antigen mutant proteins. Wild-type or mutant forms of large T antigen and small t antigen were tested in an in vitro transcription system with partially purified RNA Pol I, UBF, and SL1. GST–fusion proteins were expressed in Sf9 cells using recombinant baculovirus and purified using glutathione–Sepharose resin as described in Material and Methods. Increasing amounts of each protein [from 0.15 to 1.2 pmoles], as judged from silver-stained gels of the purified proteins, were added to the respective reaction mixtures. Transcription reactions were carried out in the presence of 100 ng of DNA plasmid pRH3, and the in vitro-synthesized transcripts were detected by S1 nuclelease protection assay as described previously [Bell et al. 1990; Comai et al. 1992]. The transcription reaction contained either no added protein [lanes 1, 5, 9, 13, 17, 21, 25, 29, 33, 37], GST protein only [lanes 2–4], GST–large T antigen [lanes 6–8], GST–large T antigen mutant proteins [lanes 10–12, 14–16, 18–20, 22–24, 26–28, 30–32, 34–36] or GST–small t antigen [lanes 38–40]. Arrows indicate the positions of the protected oligonucleotide fragments.
Discussion

Transcription of the rRNA genes by RNA Pol I is an essential process for cell homeostasis, growth, and proliferation (Jacob 1995; Moss and Stefanovsky 1995). A variety of extracellular stimuli have been shown to modulate the rate of rRNA synthesis (Jacob 1995). Following viral infection, viruses such as SV40 up-regulate the expression of the rRNA genes, and early studies have identified large T antigen as the viral protein primarily responsible for the stimulatory effect (Ide et al. 1977; Learned et al. 1983). Although well documented, relatively little was known about the mechanisms underlying this process. Most of these studies have been hampered by the lack of a well-defined RNA Pol I transcription system. With the molecular cloning of two essential Pol I transcription factors, UBF and the multiprotein complex SL1, we can now address more directly how viruses affect Pol I transcription and elucidate the molecular mechanisms of action.

In this report we focused on the biochemical analysis of the stimulation of RNA Pol I transcription by the DNA tumor virus SV40. Using an in vitro transcription system, we have reconstituted this process using recombinant large T antigen and purified Pol I transcription factors. In vitro, recombinant large T antigen stimulated RNA Pol I transcription up to fivefold, which is similar to what has been observed in vivo (this work; May et al. 1976; Pockl and Wintersburger 1980). These results suggest that the in vitro system faithfully reproduces the transcriptional activation function of large T antigen and therefore is an important tool for the identification and biochemical analysis of the cellular proteins that mediate this process.

Protein–protein interaction and immunoprecipitation experiments showed that large T antigen binds to the selectivity factor SL1, both in vivo and in vitro. This finding provides the first evidence that a viral protein such as large T antigen, through direct protein–protein interactions with SL1, modulates Pol I transcriptional activity. Moreover, analysis of large T antigen deletion mutants indicated that the amino acid region of large T antigen between residues 1 and 538 was necessary for full stimulation of Pol I transcription. A weak transcriptional stimulatory activity was retained by a T antigen mutant comprising amino acids 1–436. Further deletion into the amino terminus completely abolished the ability of large T antigen to stimulate Pol I transcription. This analysis suggests that the region between amino acids 361 and 538 is important for the stimulation of rRNA synthesis. This region of large T antigen overlaps with the ATPase domain and has also been shown to be involved in p53 binding (Fanning 1992; Conzen and Cole 1994)(Fig. 9). By binding to p53, large T antigen inactivates the antiproliferative properties of this tumor suppressor protein (Lin and Simmons 1991; Mietz et al. 1992). Thus, it is quite interesting that an overlapping domain is also required for up-regulation of Pol I transcription, and it suggests that this region is important for the proliferative program initiated by large T antigen.

The amino-terminal 362 amino acids of large T antigen, in addition to amino acids 362–538, were also necessary to reconstitute full stimulation of RNA Pol I transcription. Protein–protein interaction studies indicated that large T antigen amino acids 1–436 are required for efficient binding to TAF148, TAF110, and TBP. These results provide strong evidence that the interaction between large T antigen and SL1 plays a critical role in the stimulatory process. On the other hand, the region between amino acids 436 and 538 was also necessary for efficient stimulation of Pol I transcription, suggesting that the recruitment of large T antigen to the rRNA promoter is most likely the initial step in the activation process, and other biochemical modifications and/or changes, mediated by large T antigen, are also required. Therefore, it remains to be determined how the binding of large T antigen to SL1 influences the network of protein–protein and protein–DNA interactions between SL1, UBF, the RNA Pol I holoenzyme, and the rRNA promoter.

Figure 9. Schematic representation of SV40 large T antigen functional domains. (Top) Localization of the sequences required for the various activities, as defined by the analysis of a variety of large T antigen mutants (Fanning 1992; Conzen and Cole 1994). (Bottom) Region of large T antigen required for SL1 binding and stimulation of Pol I transcription (as described in this work).
Interestingly, our experimental results correlate well with previous work on the reactivation of rRNA genes in mouse–human hybrid cells (Soprano et al. 1979, 1981, 1983). In these studies, wild-type and mutant large T antigens were either microinjected or transfected into a mouse–human hybrid cell line that contained both mouse and human rRNA genes, but only expressed the mouse rRNA. When expressed in these cells, large T antigen could reactivate the human rRNA gene(s). In addition, this study indicated that T antigen polypeptides extending from the amino terminus to amino acid 509 or further, were active in rRNA reactivation, whereas those shorter than 420 amino acids were inactive (Soprano et al. 1983). Thus, it appears that the region of large T antigen, which is involved in the stimulation of rRNA synthesis in vitro, is also necessary for reactivation of human rRNA genes in the mouse–human hybrid cells. A question is then raised by the observation that the human rRNA gene. The cloning of the mouse SL1 subunits is multiprotein complexes comprised of TBP and polypeptide-specific TAFs (Hernandez 1993; Roeder 1996; Verrijzer and Tjian 1996). Thus, we postulate that the TBP/TAF complexes could serve as the common cellular target used by small DNA viruses such as SV40 to modulate the transcriptional activity of RNA Pol II, and, possibly, Pol III genes. Whether or not the interactions of large T antigen with TBP–TAFs are conserved between the TFIID and SL1 complex is currently unclear. We have shown that large T antigen, in addition to binding to TBP, makes direct contacts with TAF48 and TAF110. The study by Damania and Alwine (1996) indicated that TBP, TAF48, TAF110, and TAF40 are involved in the interactions between TFIID and large T antigen. However, in both studies, the specific protein–protein interaction domains in TBP and TAFs have not been mapped yet. TBP appears to be the only common target in the two complexes. Lack of homology between any of the Pol I- and Pol II-specific TAFs suggests that each of the TAFs in SL1 and TFIID interacts with large T antigen through different surfaces or that large T antigen recognizes a tertiary structure that is conserved between SL1 and TFIID. Therefore, in future work, it will be important to define the contact points between large T antigen and TAFs in the TFIID and SL1 complexes. Ultimately, structural studies of TFIID and SL1 should provide the necessary framework to address these issues.

Materials and methods

Plasmids and DNA constructs

Large T antigen cDNA was released from pNT2 (Lanford 1988) by cleavage with BamHI and ligated into BamHI-digested pBluescriptIIKS(+) in T7 orientation. A 2.3-kb HindIII partially digested fragment released from this plasmid was then ligated into pBluescriptIIKS(+) (HI) digest to generate pBSK5-T(ΔTAG) with T antigen in T7 orientation. Baculovirus-expressing vectors pVL2T and pVL3X were generated by religating Small/EcoRI-digested pVL1393 (Pharmpingen) with a 0.75-kb HindII–EcoRI fragment released from pGEX2T and pGEX3X (Pharmpigen), respectively. pVL3X–WT-T was generated by ligating a 2.3-kb EcoRI fragment released from pBSK5–T(ΔTAG) into pVL3X/EcoRI. pVL3X–TN1 was generated by ligating a 1.7-kb EcoRI–PstI fragment from pBSK5–T(ΔTAG) into PstI–EcoRI-digested pVL3X. pVL2T–TN2 was constructed by ligating a 1.4-kb Small/PvuII fragment from pBSK5–T(ΔTAG) into Small digested pVL2T. pVL2T–TN3 was generated by ligating a 1.1-kb Small–HindII fragment from pBSK5–T(ΔTAG) into Small-digested pVL2T. pVL2T–TC1 was generated by ligating a 1.3-kb Small–HindII fragment from pBSK5–T(ΔTAG) into Small-digested pVL2T. pBSK5–TC2 was constructed by ligating a 1.0-kb NsiI–EcoRI fragment from pBSK5–T(ΔTAG) into PstI–EcoRI-digested pBluescriptIIKS(+) (Roeder, personal communication). pVL2T–TC2 was generated by ligating an 1.0-kb Small fragment from pBSK5–TC2 into Small-digested pVL2T. pVL2T–TC3 was generated by ligating a 0.7-kb PstI fragment from pBSK5–T(ΔTAG) into PstI-digested pVL2T. pVL2T–TM1 was generated by self-ligating the bigger fragment from PstI-digested pVL2T–TC1. For small t antigen constructs, small t cDNA was released from pSVO10, a plasmid containing the SV40 genome. A 1.9-kb PvuII fragment released from pSVO10 (gift from B. Stillman, Cold Spring Harbor Laboratory) was digested with HindIII to produce a 1.1-kb fragment containing small t cDNA. The fragment containing small t cDNA was ligated into HindIII-digested pBluescriptIIKS(+) to generate pBSK5–small t. pVL2T–small t was generated by ligating a 1.2-kb Small–HindII fragment from pBSK5–small t into Small-digested pVL2T. The plasmids pCMV-LT and pCMV-asT were generated by insertion of BamHI large T antigen DNA fragment into pCBB6A3 [Invitrogen], in both orientations.

Plasmid pHu3 containing the human rRNA promoter and the plasmids containing the human TAFs and TBP have been described previously (Bell et al. 1988; Comai et al. 1992, 1994). pHu3CAT was constructed by inserting the human rDNA promoter (–500 to +78) into pBSMCAT (CAT cDNA inserted in pBS, gift of Dr. S. Tahara, University of Southern California, Los Angeles) at the StuI site. The contract was sequenced to ensure that the promoter was placed in the correct orientation.

Cell culture, transfections, and SV40 infections

HeLa cells (strain S3) were propagated and maintained in minimal essential medium (MEM) supplemented with 5% newborn calf serum at 37°C in 5% CO2. Cells were kept in a spinner at a density of 2 x 10^6 to 5 x 10^6 cells/ml. For infection with SV40, 1 liter of HeLa cells was collected by spinning at 1000g for 5 min and cell pellets were resuspended in 10 ml of MEM and 10 ml of SV40 virus. Infection was done at a m.o.i of 20 PFU/cell and incubated for 2 hr at 37°C with periodic rocking. Then fresh medium was added and cells were incubated for 48 hr at 37°C. Infected and mock-infected cells were harvested, and nuclear
extracts were prepared as described in Comai et al. [1992]. The protein concentration of the extracts was determined by the Bradford assay.

For the transfection assays, HeLa cells were maintained in DMEM supplemented with 10% FBS at 37°C with 5% CO₂. Cells were seeded at 7.5 x 10⁵ per 100-mm plate 1 day prior to transfection. Cells were transfected by the calcium phosphate method with the indicated amounts of plasmid DNA. Filler DNA (pBS) was used to normalize the amount of DNA used per transfection. Cells were harvested 56-60 hr after transfection, and total RNA was isolated using the guanidinium–thiocyanate method. Thirty micrograms of total RNA was then used in a standard primer extension assay with a CAT-specific primer. The cDNA product was resolved on an 8% polyacrylamide–urea gel and subjected to autoradiography. Quantitation was performed using a PhosphorImager (Molecular Dynamics). A 60-nucleotide 32P-end-labeled oligonucleotide was included in all of the reaction mixtures to account for sample losses during the experimental procedure.

Sf9 cells were propagated and maintained in Hink’s TNM–FH insect medium [IRH Biosciences] supplemented with 10% heat-inactivated fetal bovine serum at 28°C. To generate recombinant baculoviruses, Sf9 cells were seeded on a six-well tissue culture plate to ~80% confluency and allowed to attach for 30 min. Cells were then transfected with 1.3 μg of baculovirus expression construct together with 0.17 μg of BaculoGold DNA as described previously (Lanford 1988). Sf9 cells infected with recombinant baculovirus were harvested 42-48 hr postinfection and washed with 1 x phosphate-buffered saline (PBS). Cell pellets were resuspended in TM buffer containing 0.4 M KC1 and the TAFs were eluted at 0.5 M KC1. Fractions containing TAF were then dialyzed in TM buffer containing 0.1 M KC1, and the concentration of TAFs was estimated from silver-stained SDS–polyacrylamide gels. All of the buffers were supplemented with 1 mM PMSF, 1 mM sodium metabsulfite, 5 μg/ml of leupeptin, and 5 μg/ml of aprotinin.

**Purification of GST fusion proteins**

GST–large T antigen and GST–large T antigen mutant proteins were expressed in Sf9 cells using recombinant viruses. Cell lysates were incubated with glutathione–affinity resin [Pharmacia] for 1 hr at 4°C. The beads were then washed five times with TM/0.2 M KC1 plus 0.5% NP-40 followed by two times with elution buffer [0.1% Triton X-100, 50 mM NaCl, 0.1% NP-40]. GST–fusion proteins were eluted from the beads using 20 mM reduced glutathione dissolved in elution buffer. Eluted proteins were dialyzed against TM/0.1 M KC1. The amount of eluted protein was estimated by Coomassie and silver staining of SDS–polyacrylamide gels.

**Purification of Flag-tagged TAFs**

Flag-tagged TAF110, TAF63, and TAF48 were expressed individually in insect cells as described previously (Comai et al. 1994). Each one of the TAFs was purified from the respective cytoplasmic fraction as follows. Cytoplasmic extracts were prepared by lysing the cells in buffer H [10 mM Tris-HCl (pH 7.9), 10 mM KC1, 1.5 mM MgCl₂, 1 mM DTT] using a Dounce homogenizer. The extract was then adjusted to 0.1 M KC1, loaded on a DEAE-Sepharose column, and step-eluted with TM buffer containing 0.3, 0.5, 0.7, and 1.0 M KC1. TAF63 and TAF48 eluted at 0.3 M KC1, and TAF110 eluted at 0.5 M KC1. Fractions containing TAF were then loaded onto an anti-Flag M2–affinity resin [Kodak], washed extensively with TM buffer containing 0.4 M KC1, and the TAFs were eluted with TM buffer/0.1 M KC1 containing 0.4 mg/ml of Flag peptide. The eluted proteins were then dialyzed in TM/0.1 M KC1, and the concentration of TAFs was estimated from silver-stained SDS–polyacrylamide gels. All of the buffers were supplemented with 1 mM PMSF, 1 mM sodium metabsulfite, 5 μg/ml of leupeptin, and 5 μg/ml of aprotinin.

**Purification of GST-E1A-13S**

GST fusion protein was expressed in *Escherichia coli* and affinity purified on glutathione–Sepharose beads as described above. For transcription assays, from 50 to 150 ng of purified protein was added to each reaction, as estimated by silver-stained SDS–polyacrylamide gels.

**In vitro transcription assay**

In vitro transcription reactions were carried out as described previously in the presence of 100 μg/ml of α-amanitin (Comai et al. 1992). In vitro-synthesized RNAs were detected by S1 nuclease analysis using 5‘-end labeled single-strand DNA oligonucleotides. In the presence of wild-type and mutant large T antigen proteins, transcription was carried out by preincubating the large T antigen protein with a transcription mixture without the four ribonucleotides on ice for 20 min. After the incubation, all four ribonucleotides were added to initiate the transcription reaction.

**Protein–protein interaction assays**

Sf9 cells infected with recombinant baculovirus were harvested 42-48 hr postinfection and washed with 1 x phosphate-buffered saline [PBS]. Cell pellets were resuspended in TM/0.1 M KC1 buffer and lysed by sonication. NP-40 was then added to cell lysates to a final concentration of 0.1%. Cell lysates were cleared by centrifugation at 30,000g for 30 min. The amount of GST–fusion proteins used in the binding reactions was estimated by Bradford assays and Coomassie staining of SDS–polyacrylamide gels. Equal molar amounts of each GST–fusion protein were used in the interaction assays. All binding reactions were performed at 4°C with constant mixing on a nutator. Glutathione beads were allowed to bind the GST–fusion proteins for 1 hr in TM/0.1 M KC1 plus 0.1% NP-40. The beads were then washed four times with the binding buffer. Human TAFs were synthesized using the TNT-coupled in vitro transcription system.
Purification of protein from HeLa cells

Nuclear extracts from HeLa cells were prepared as described (Comai et al. 1992). They were loaded onto a heparin-agarose column and RNA Pol I, UBF, and SLI were eluted with a 0.1–1.0 M KCl gradient. Fractions containing SL1 activity, as determined by in vitro transcription assays, were pooled and dialyzed against TM°/0.1 M KC1. RNA Pol I used in the transcription assays was purified as described previously (Comai et al. 1992). UBF was either partially purified by chromatography on heparin-agarose, DEAE, and Q-Sepharose, or purified to homogeneity (Bell et al. 1988). The two preparations of UBF were used interchangeably in the transcription assays, with identical results. Although RNA Pol I and SLI were purified partially, they were not cross-contaminated with each other or UBF.

For the in vivo co-immunoprecipitation analysis, nuclear extracts from SV40-infected and mock-infected HeLa nuclear extracts were prepared as described above with the exception that the KCl concentration in the nuclear extracts was brought to 0.4 M before loading onto a heparin-agarose column pre-equilibrated in TM°/0.4 M KCl. After washing extensively with TM°/0.4 M KCl buffer, the SL1 fraction was step eluted with TM°/0.8 M KCl buffer and dialyzed against TM°/0.1 M KCl.

Co-immunoprecipitation from SV40-infected HeLa cells and Western blot analysis

SL1 from either SV40-infected or mock-infected HeLa cell nuclear extracts was prepared as described previously. The SL1 purified from 100 ml of either SV40-infected or mock-infected HeLa cells was used in each assay. For the co-immunoprecipitation, anti-large T antigen monoclonal antibody (pAb 101) was incubated with 30 µl of a 50% (vol/vol) slurry of protein A-Sepharose beads for 1 hr at 4°C. The beads were washed five times with TM°/0.1 M KCl containing 0.1% NP-40 and then mixed with the SL1 fraction for 8 hr at 4°C with constant mixing on a nutator. After washing with the binding buffer five times, the protein was eluted with 1.0 M guanidine-hydrochloride in TM°/0.1 M KCl. Elution was performed twice for 10 min each in 50 µl at 4°C. Eluate was combined and diluted with 3 volumes of TM°/0.1 M KCl. Proteins were then precipitated with TCA for 20 min on ice and collected by centrifugation at 30,000g for 20 min. Pellets were washed twice with cold acetone and air-dried. Samples were boiled in SDS sample buffer, resolved by SDS-PAGE, and transferred to nitrocellulose membrane. The SL1 subunits were detected by incubation with the appropriate antibody [either α-TAF48, α-TAF63, α-TAF110, or α-TBP] and visualized by using either the ECL chemiluminescence procedure (Pierce) or the alkaline-phosphatase method following reaction with NBT and BCIP (Harlow and Lane 1988).

Acknowledgments

We thank Robert Lanford for the large T antigen baculovirus expression vector pNT2, Bruce Stillman for SV40 virus and plasmid pSV010, Brian Dynlacht for the E1A-13S expression construct, and Stanley Tahara for pBSMCAT plasmid. We are grateful to M. Lai, A. Lee, A. Schonthal, D. Johnson, and members of their laboratories for helpful advice. We thank Luca Comai, P. Verrijzer, R. Wilds, and J. Sarabia for critically reading the manuscript. This work was supported, in part, by an American Cancer Society Institutional Research grant (IRG-21-35) and a James H. Zumberge Faculty Research and Innovation Fund (L.C.). W.Z. was supported, in part, by a Kenneth Norris, Jr., Comprehensive Cancer Center graduate fellowship.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Beckmann, H., J.-L. Chen, T. O’Brien, and R. Tjian. 1995. Coactivator and promoter-selective properties of RNA polymerase I TAFs. Science 270:1506–1509.

Bell, S.P., R.M. Learned, H.-M. Jantzen, and R. Tjian. 1988. Functional cooperativity between transcription factors UBF1 and SL1 mediates human ribosomal RNA synthesis. Science 241:1192–1197.

Bell, S.P., H.-M. Jantzen, and R. Tjian. 1990. Assembly of alternative multiprotein complex directs rRNA promoter selectivity. Genes & Dev. 4:943–954.

Berger, L., D.B. Smith, J. Davidson, J.-H. Hwang, E. Fanning, and A.G. Wildeman. 1996. Interaction between T antigen and TEA domain of the factor TEF-1 derepresses Simian virus 40 late promoter in vitro: Identification of T-antigen domains important for transcriptional control. J. Virol. 70:1203–1212.

Cavanaugh, H.A. and E.A. Thompson, Jr. 1986. Hormonal regulation of transcription of rDNA. J. Biol. Chem. 261:12738–12744.

Clark, R., K. Peden, J.M. Pipas, D. Nathans, and R. Tjian. 1983. Biochemical activities of T-antigen proteins encoded by simian virus 40 gene deletion mutants. Mol. Cell. Biol. 3:220–228.

Clos, J., D. Buttgereit, and I. Grummt. 1986. A purified transcription factor (TIF-B) binds to essential sequences of the mouse rDNA promoter. Proc. Natl. Acad. Sci. USA 83:604–608.

Comai, L., N. Tanese, and R. Tjian. 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., J.C.B.M. Zomerdijk, H. Beckmann, S. Zhou, A. Admon, and R. Tjian. 1994. Reconstitution of transcription factor SL1: Exclusive binding of TBP by SL1 or TFIIID subunits. Science 266:1966–1972.

Conzen, S.D. and C.N. Cole. 1994. The transforming proteins of simian virus 40. Semin. Virol. 5:349–356.

Damaia, B. and J.C. Alwine. 1996. TAF-like function of SV40 large-T antigen. Genes & Dev. 10:1369–1381.

Eberhard, D., L. Tora, J.M. Egly, and I. Grummt. 1993. A TBP-containing multiprotein complex (TIF-B) mediates transcription specificity of murine RNA polymerase I. Nucleic Acids Res. 21:4180–4186.

Fanning, E. 1992. Simian virus 40 large T antigen: The puzzle, the pieces, and the emerging picture. J. Virol. 66:1289–1293.

Gruda, M.C., J.M. Zabolotny, J.H. Xiao, I. Davidson, and J.C. Alwine. 1993. Transcriptional activation by simian virus 40 large T antigen: Interaction with multiple components of the
transcription complex. **Mol. Cell. Biol.** 13: 961–969.

Grummt, I., A. Smith, and F. Grummt. 1976. Amino acid starvation affects the initiation frequency of nucleolar RNA polymerase. **Cell** 7: 439–445.

Hammond, L.M. and H.L. Bowman. 1988. Insulin stimulates the translation of ribosomal proteins and the transcription of rRNA in mouse myoblast. **J. Biol. Chem.** 263: 17785–17791.

Harlow, E. and D. Lane. 1988. **Antibodies: A laboratory manual.** Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? **Genes & Dev.** 7: 1291–1308.

Hinds, P.W. and R.A. Weinberg. 1994. Tumor suppressor genes. **Curr. Opin. Genet. Dev.** 4: 135–141.

Jantzen, H.-M., A.M. Chow, D.S. King, and R. Tjian. 1992. Multiple domains of the RNA polymerase I activator hUBF interacts with the TATA-binding protein complex hSL1 to mediate transcription. **Genes & Dev.** 6: 1950–1963.

Johnston, S.D., X.-M. Yu, and J.E. Mertz. 1996. The major transcriptional transactivation domain of Simian virus 40 large T antigen associates nonconcurrently with multiple components of the transcriptional preinitiation complex. **J. Virol.** 70: 1191–1202.

Jantzen, H.-M., A. Admon, S.P. Bell, and R. Tjian. 1990. Nuclear transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. **Nature** 344: 830–836.

Jantzen, H.-M., A.M. Chow, D.S. King, and R. Tjian. 1992. Multiple domains of the RNA polymerase I activator hUBF interacts with the TATA-binding protein complex hSL1 to mediate transcription. **Genes & Dev.** 6: 1950–1963.

Learned, R.M., T.K. Learned, M.M. Haltiner, and R. Tjian. 1996. The major transcriptional transactivation domain of Simian virus 40 large T antigen associates nonconcurrently with multiple components of the transcriptional preinitiation complex. **J. Virol.** 70: 1191–1202.

Learned, R.M., S.T. Smale, M.M. Haltiner, and R. Tjian. 1983. Regulation of human ribosomal RNA transcription. **Proc. Natl. Acad. Sci.** 80: 3558–3562.

Learned, R.M., S. Cordes, and R. Tjian. 1985. Purification and characterization of a transcription factor that confers promoter specificity to human RNA polymerase I. **Mol. Cell. Biol.** 5: 1358–1369.

Learned, R.M., T.K. Learned, M.M. Haltiner, and R. Tjian. 1986. Human rRNA transcription is modulated by the coordinate binding of two factors to an upstream control element. **Cell** 45: 847–857.

Lin, J.-Y. and D.T. Simmons. 1991. The ability of large T antigen to complex with p53 is necessary for the increased life span and partial transformation of humna cells by simian virus 40. **J. Virol.** 65: 6447–6453.

May, P., E. May, and J. Borde. 1976. Stimulation of cellular RNA synthesis in mouse-kidney cell cultures infected with SV40 virus. **Exp. Cell Res.** 106: 433–439.

Miesfeld, R., B. Sollner-Webb, C. Croce, and N. Arnheim. 1984. The absence of a human-specific ribosomal DNA transcription factor leads to nucleolar dominance in mouse greater than human hybrid cells. **Mol. Cell. Biol.** 4: 1306–1312.

Mietz, J.A., T. Unger, J.M. Huibregtse, and P.M. Howley. 1992. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T antigen and by HPV-16 E6 oncoprotein. **EMBO J.** 11: 5013–5020.

Mishima, Y.M. and M. Muramatsu. 1979. The mechanism of decrease in nucleolar RNA synthesis by protein synthesis inhibitor. **J. Biochem.** 85: 807–818.

Mishima, Y.M., L. Financsek, R. Dominami, and M. Muramatsu. 1982. Fractionation and reconstitution of factors required for accurate transcription of mammalian ribosomal RNA genes: Identification of a species-dependent factor. **Nucleic Acids Res.** 10: 6659–6670.

Moss, T. and V.Y. Stefanovsky. 1995. Promotion and regulation of ribosomal transcription in eukaryotes by RNA polymerase I. **Progr. Nucleic Acids Res. Mol. Biol.** 50: 25–66.

Nevin, J.R. 1994. Cell cycle targets of the DNA tumor viruses. **Curr. Opin. Genet. Dev.** 4: 130–134.

Pockl, E. and E. Wintersburger. 1980. Increased rate of RNA synthesis: Early reaction of primary mouse kidney cells to infection with polyoma virus or simian virus 40. **J. Virol.** 35: 8–19.

Reeder, R.H. 1994. **Regulation of transcription by RNA polymerase I.** Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Roeder, R.G. 1986. The role of general initiation factors in transcription by RNA polymerase II. **Trends Biochem. Sci.** 21: 327–334.

Sakrany, G., N. Tanaka, T. Kishimoto, Y. Ishikawa, H. Kato, R. Kominami, and M. Muramatsu. 1989. Structural determinant of the species-specific transcription of the mouse rRNA gene promoter. **Mol. Cell Biol.** 9: 349–353.

Schnapp, A., H. Rosenbauer, and I. Grummt. 1991. Trans-acting factors involved in species-specificity and control of mouse ribosomal gene transcription. **Mol. Cell. Biol.** 104: 137–147.

Simanis, V. and D.F. Lane. 1985. An immunopurification procedure for SV40 Large-T antigen. **Virology** 144: 88–100.

Sollner-Webb, B. and J. Tower. 1986. Transcription of cloned eukaryotic ribosomal RNA genes. **Annu. Rev. Biochem.** 55: 801–830.

Sommerville, J. 1986. Nucleolar structure and ribosome biogenesis. **Trends Biochem. Sci.** 11: 438–442.

Soprano, K.J., V.G. Dev, C.M. Croce, and R. Baserga. 1979. Reactivation of silent rRNA genes by simian virus 40 in human–mouse hybrid cells. **Proc. Natl. Acad. Sci.** 76: 3885–3889.

Soprano, K.J., G.J. Jonak, N. Galanti, J. Floros, and R. Baserga. 1981. Identification of an SV40 DNA sequence related to the reactivation of silent rRNA genes in human > mouse hybrid cells. **Virology** 109: 127–136.

Soprano, K.J., N. Galanti, G.J. Jonak, S. McKercher, J.M. Pipas, K.W.C. Peden, and R. Baserga. 1983. Mutational analysis of simian virus 40 T antigen: Stimulation of cellular DNA synthesis and activation of rRNA genes by mutants with deletions in the T-antigen gene. **Mol. Cell. Biol.** 3: 214–219.

Tanese, N., B.F. Pugh, and R. Tjian. 1991. Coactivators for a proline-rich activator purified from the multisubunit human TFIIID complex. **Genes & Dev.** 5: 2212–2224.

Verrizier, C.P. and R. Tjian. 1996. TAFs mediate transcriptional activation and promoter selectivity. **Trends Biochem. Sci.** 21: 338–342.
SV40 large T antigen binds to the TBP-TAF(I) complex SL1 and coactivates ribosomal RNA transcription.

W Zhai, J A Tuan and L Comai

*Genes Dev.* 1997, 11:
Access the most recent version at doi:10.1101/gad.11.12.1605