The simian virus 40 (SV40) early gene product large T antigen (T antigen) is known to be a promiscuous activator of many viral and cellular promoters. Such promiscuity is suggested by the structural simplicity required of a promoter for activation by large T antigen, a TATA box or initiator element with at least one upstream transcription factor-binding site (which can be variable) is adequate (Gilinger and Alwine 1993; Gruda et al. 1993; Rice and Cole 1993). Although large T antigen is a known DNA-binding protein, this function is not essential for transcriptional activation (Keller and Alwine 1985; Gallo et al. 1988, 1990; Beard and Bruggmann 1989; Zhu et al. 1991; Casaz et al. 1995). Our previous studies have suggested that the activation of such promoters requires large T antigen to interact, through protein–protein interactions, with both the basal transcription complex and the upstream-bound factor (Gilinger and Alwine 1993; Gruda et al. 1993). For example, we have shown that large T antigen can interact with TATA-binding protein (TBP), transcriptional enhancer

factor-1 (TEF-1) (Gruda et al. 1993) and Sp-1 (B. Damania and J.C. Alwine, unpubl.). It is important to note that although large T antigen can interact with a component of TFIID, it cannot activate a promoter containing only a TATA element. The additional interaction with an upstream-bound factor appears to be essential for activation. Such a mechanism of activation is similar to that of the TBP-associated factors (TAFs). These components of TFIID cannot mediate transcriptional activation unless they interact with upstream-bound factors (Hoey et al. 1993; Chen et al. 1994). These similarities in function raise the question of whether large T antigen may perform a TAF-like function.
Ruppert et al. 1993). In the temperature-sensitive (ts) hamster cell line ts13 [Talavera and Basilico 1977] the ts defect results from mutation of CCG (Sekiguchi et al. 1988; Hayashida et al. 1994; Noguchi et al. 1994). The ts defect in ts13 cells was first characterized as a cell cycle defect because the cells arrest in G1 at the nonpermissive temperature [Talavera and Basilico 1977]. However, the ts defect in TAf250 can be noted at the transcriptional level [Liu et al. 1985; Wang and Tjian 1994]. At the nonpermissive temperature, ts13 cells do not exhibit a global defect in transcription, but transcription of specific genes is dramatically decreased [Wang and Tjian 1994]. For example, the activity of the cyclin A (cycA) promoter is decreased by 8- to 10-fold at the nonpermissive temperature compared to its activity at the permissive temperature; however, the activity of the fos promoter is relatively unaffected by the temperature [Wang and Tjian 1994]. Stable transfection of the TAf250 gene into ts13 cells rescues the ts defect in transcriptional activation [Wang and Tjian 1994].

Previously, studies of the cell cycle phenotype of the ts defect in ts13 cells indicated that the introduction of T antigen overcame the G1 arrest [Floros et al. 1981]. This suggested to us that T antigen may be able to rescue the ts transcriptional defect in TAf250. In this study, we show that SV40 large T antigen can rescue the transcriptional defect of TAf250 in ts13 cells at the nonpermissive temperature. In addition, we show that large T antigen and TAf250 share common in vitro interactions with TAFs and TBP, and that large T antigen communoprecipitates with TFII D from infected cell extracts. In addition, we show that large T antigen copurifies with TFIID over phosphocellulose and communoprecipitates with the purified TFIID. Mutants in large T antigen defective in transcriptional activation neither rescued the ts defect in TAf250 nor communoprecipitated with TFIID. We conclude from these findings that large T antigen performs a TAF-like function in a complex with TFIID.

Results

T antigen, like TAf250, can rescue the transcriptional defect in ts13 cells

In previous studies of the ts defective in ts13 cells was studied using the cycA promoter [Wang and Tjian 1994]. Hence we used the same cycA–luciferase reporter plasmid in these studies. In the following transfection studies we asked whether T antigen, like wild-type TAf250, could rescue the ts defect of TAf250 in ts13 cells.

Figure 1 shows the results of transfection of ts13 cells with the cycA reporter plasmid either alone or with increasing amounts of a plasmid expressing T antigen (bottom) or Ela (top). In both experiments it can be seen that at the nonpermissive temperature (39°C) the cycA promoter alone (0 effector plasmid point) showed ~10% of the activity seen at the permissive temperature (32°C). This is in agreement with similar studies by Wang and Tjian [1994]. The addition of the Ela-expressing plasmid showed that Ela can activate the cycA promoter at the permissive temperature. Importantly, however, Ela failed to activate the promoter at the nonpermissive temperature. Conversely, T antigen caused very little activation at the permissive temperature; in numerous experiments the greatest activation mediated by large T antigen was <2.5-fold at 32°C. However, the more significant observation is that large T antigen activated the cycA promoter at the nonpermissive temperature, increasing its activity to a level approximately equal to that at the permissive temperature. These data suggest that T antigen and Ela are activating by very different mechanisms (see Discussion) and that T antigen, and not Ela, can rescue the ts defect in TAf250.

Figure 2 shows the results of experiments with ts13 cells at the nonpermissive temperature where the cycA-luciferase reporter plasmid were transfected either alone (with filler plasmid) or with the indicated amounts of effector plasmids that expressed either Ela or large T antigen. Transfections and assays were done as described in Materials and methods.
cycA promoter activity at 32°C where the activity at 32°C is set as 100%. This provides a comparative display of the ability of the activators to restore cycA promoter activity at the nonpermissive temperature. As noted in Figure 1, at the nonpermissive temperature, the activity of the cycA promoter alone fell to 10%-15% of the activity of the promoter at the permissive temperature. The addition of the TAFii250-expressing plasmid rescued the activity of the cycA promoter to the level seen at the permissive temperature. This agrees well with the data of Wang and Tjian (1994) showing that TAFii250 restored the activity of the cycA promoter in ts13 cells at the nonpermissive temperature. Similarly, the addition of large T antigen rescued the promoter activity to ~90% of the activity at the permissive temperature. However, neither Ela nor small t antigen affected the activity of the cycA promoter at the nonpermissive temperature. Interestingly, the combination of both large T antigen and small t antigen produced a synergistic activation. This is shown in two experiments in Figure 2 where (1) large T antigen and small t antigen were introduced on separate plasmids (T + t Sep.), and (2) a single plasmid was transfected encoding the entire early region (ER), which can produce both large T antigen and small t antigen by alternatively splicing the early transcript (T + t ER).

In a similar experiment, mutants of large T antigen that fail to bind either p53 (Bentivoglio et al. 1992) or retinoblastoma gene product (Rb) (Kaelin et al. 1990) were tested [data not shown]. The results suggested that failure of large T antigen to bind either p53 or Rb had no dramatic effect on the ability of large T antigen to activate the cycA promoter at the nonpermissive temperature. This agrees with previous results that showed that p53 and Rb binding were not essential for transcriptional activation (Zhu et al. 1991) and data that showed that transcriptional activation by large T antigen was not altered in cells that lacked both Rb and p53 (Trifillis et al. 1990).

As mentioned in the introductory section, the results of Wang and Tjian (1994) indicated that the ts transcriptional defect in TAFii250 in ts13 cells was promoter specificity, that is, the cycA promoter was affected whereas the fos promoter was not. To test whether T antigen showed a similar specificity in ts13 cells we asked whether the presence of large T antigen would affect the activity of the fos promoter at the nonpermissive temperature. In transfection experiments similar to those described above, we noted that the activity of the fos promoter alone increased approximately twofold at the nonpermissive temperature relative to the permissive temperature. Cotransfection of increasing amounts of the large T antigen expression plasmid (1, 3, 5 μg) provided no additional activation [data not shown]. This suggests similar promoter specificity between large T antigen and TAFii250, in agreement with the interpretation that large T antigen provides a function in ts13 cells similar to TAFii250.

Trans-activation mutants of large T antigen fail to rescue the ts defect in TAFii250

To establish that large T antigen’s ability to rescue the ts defect in TAFii250 correlated with defined trans-activation functions of large T antigen we tested a number of mutants that have been characterized previously for trans-activation (Zhu et al. 1991). The mutants tested [small in-frame deletions and insertions, Table 1] all
make full-length or near full-length large T antigen. Zhu et al. [1991] determined the ability of each mutant to activate the SV40 late promoter in CV-1 cells. In Figure 3A these late promoter trans-activation data (TA LP) are plotted along with our determination of the effect of each mutant (5 µg of transfected plasmid) on cycA promoter activity in ts13 cells at 39°C (TA CYC39); promoter activity in all cases is expressed as the percentage of the activation by wild-type T antigen where wild type is 100%. Each promoter was similarly affected by the mutants suggesting that the activation of the cycA promoter in ts13 cells at 39°C correlates with a defined trans-activation function of large T antigen.

In Figure 3B the activation of the CycA promoter in ts13 cells at 39°C is expressed as the percentage of the cycA promoter activity at 32°C, similar to the comparison in Figure 2. As seen previously, the basal activity of the cycA promoter at 39°C fell to a level of 10%-15% of the activity at 32°C. In this set of experiments wild-type large T antigen restored the activity of the promoter to 80% of the activity at 32°C. Mutants inA2815 and inA2835, previously shown to be the most defective in transcriptional activation, are the most defective for rescue of the TAFn250 ts mutation.

Interactions of TAFs with SV40 T antigen

The above data show that T antigen can rescue the ts defect of TAFn250 in ts13 cells at the nonpermissive temperature. This suggests that large T antigen may function in a manner similar to a TAF, specifically TAFn250. One of the characteristics of TAFn250 is its ability to interact with TBP and other TAFs, acting as a scaffolding protein for the formation of TFIID. To determine whether large T antigen can interact with components of TFIID, we used a group of glutathione-S-transferase fusion proteins (Fig. 4) with full-length large T antigen [GST-FLT] and portions of it (T1-T5) to assay binding to in vitro transcribed and translated 35S-labeled dTAFn250, dTAFn150, dTAFn110, dTAFn80, dTAFn60, dTAFn40 [Hoey et al. 1993]. It should be noted that we have tried to make larger portions of the carboxy-termi-

Table 1. Large T antigen mutants tested

| Mutant     | Type of mutation | Region/domain affected   |
|------------|------------------|-------------------------|
| inA2803    | insertion at amino acid 34 | amino terminus          |
| inA2811    | insertion at amino acid 424 | ATP-binding and ATPase domain |
| inA2815    | insertion at amino acid 168 | DNA-binding domain      |
| inA2817    | insertion at amino acid 219 | DNA-binding domain      |
| dl2831     | deletion of amino acids 4-34 | amino terminus          |
| inA2835    | insertion at amino acids 85 and 86 | amino terminus          |

Figure 3. (A) Mutants of T antigen characterized previously for transactivation of the cycA promoter in ts13 cells at 39°C. Five micrograms of the respective plasmids (Table 1) were transfected and luciferase activity assayed as described in Materials and methods. The trans-activation of the cycA promoter (TA CYC39; hatched bars) is shown in comparison with the data of Zhu et al. [1991] for the trans-activation of the SV40 late promoter (TA LP; solid bars). Activation of each promoter by wild-type large T antigen is set at 100%. (B) The activation of the cycA promoter at 39°C is expressed as the percentage of the activity of the cycA promoter alone at 32°C, similar to the representation of the data in Fig. 2. The shaded bars on top indicate standard deviation.

Figure 5A shows the results of the binding analyses (lanes; In = input; G = GST; 1 = GST-T1; 2 = GST-T2; 3 = GST-T3; 4 = GST-T4; 5 = GST-T5; T = GST-FLT; t = GST-t). Because exposure times are variable between the various experiments, the data were quantitated by PhosphorImager analysis and presented in Figure 5B as the percentage input TAF bound after subtraction of the nonspecific binding to the GST moiety. The data suggest that full-length large T antigen bound significantly to dTAFn150, dTAFn110, and dTAFn40. In experiments us-

Table 1. Large T antigen mutants tested
TAF-like function of T antigen

Figure 4. Diagram of full-length T antigen and regions of large T antigen that were fused to the glutathione binding site of glutathione-S-transferase (GST). The amino acids included in each GST fusion are indicated along with the regions name; FLT (full length T antigen), T1, T2, T3, T4, and T5.

Figure 5. [A] The results of binding of dTAFn250, dTAFn150, dTAFn110, dTAFn80, dTAFn60, and dTAFn40, to GST fusions of large T antigen and small t antigen. Lane “In” of each lane shows the total input of in vitro transcribed and translated 35S-labeled TAFs used in each binding reaction. Each labeled TAF was bound to the GST moiety alone (lane G) as well as to the various GST fusions with T antigen shown in Fig. 4: (lane 1) GST-T1, (lane 2) GST-T2, (lane 3) GST-T3, (lane 4) GST-T4, (lane 5) GST-T5, (lane 7) GST-full length T. In addition, lane “t” shows the binding to a GST fusion with small t antigen. [B] The binding data in A were quantitated using a Molecular Dynamics PhosphorImager. After subtraction of background binding to the GST moiety alone, the percentage of input TAF bound was calculated and plotted.

Coimmunoprecipitation of T antigen with complexes containing TBP

Previously, we have shown that TBP can bind to GST-FLT, GST-T1, and GST-T5 in vitro [Gruda et al. 1993]. These data and the data above suggest that large T antigen may interact with a number of the components of TFIID. To confirm that such interactions occur in vivo, we used α3 cells, a HeLa cell line that expresses consti-
tutively an influenza hemagglutinin (HA) epitope-tagged TBP [eTBP; Zhou et al. 1992]. Immunoprecipitation of \(\alpha_3\) cell nuclear extracts with anti-HA antibody has been shown to precipitate holo-TFIIID [Zhou et al. 1992]. To test whether T antigen is associated with TFIIID in vivo we used SV40 [strain 776] to infect normal HeLa cells (HeLa/SV40) or \(\alpha_3\) cells (\(\alpha_3\)/SV40) at an m.o.i. of 20 pfu/cell. Forty-eight hours after infection, the cells were harvested and nuclear extracts were prepared and analyzed in a variety of immunoprecipitation experiments described below (see also Material and methods).

Figure 6 shows the coimmunoprecipitation of eTBP and large T antigen using either the anti-HA antibody or an anti-TBP antibody. Nuclear extract was mixed with antibodies and precipitated with protein A–Sepharose beads. The immunoprecipitates were separated by SDS-PAGE and transferred to nitrocellulose. The resulting Western blot was probed with anti-T antigen antibody to detect the presence of T antigen in the immunoprecipitates. Lanes 6 and 7 show 50 \(\mu\)g of the HeLa/SV40 and \(\alpha_3\)/SV40 unprecipitated cell extracts. The presence of SV40 large T antigen can be seen migrating at \(-92\) kD, similar extracts from noninfected cells showed no band at this position [not shown]. Lanes 4 and 5 show that both the anti-HA and anti-TBP immunoprecipitates from the \(\alpha_3\)/SV40 extracts coprecipitated T antigen. Conversely, control immunoprecipitates from extracts of noninfected \(\alpha_3\) cells (\(\alpha_3\)) showed no T antigen (lanes 2 and 3). An additional control in lane 1 shows that the anti-HA antibody precipitated no T antigen from the HeLa/SV40 extracts indicating no cross-reactivity between the anti-HA antibody and large T antigen. The results confirm that the large T antigen detected in the immunoprecipitates from the \(\alpha_3\)/SV40 extracts was present because of coprecipitation in a complex with TBP or TFIIID. The bands at \(-50\) kD are the heavy chains of the antibodies used for the immunoprecipitation. These are present in large amounts and cross-react with the secondary antibody used to probe the blot.

To verify the coprecipitation of large T antigen and TBP or TFIIID, we performed the reverse immunoprecipitation where anti-T antigen antibody was used to precipitate the cell extracts and the resulting Western blots were examined for coprecipitated eTBP using the anti-HA antibody. Figure 7 shows the results of such analysis on uninfected \(\alpha_3\) cell extract, \(\alpha_3\)/SV40 extract, and HeLa/SV40 extract. Comparisons of lanes 4, 5, and 6 show that the anti-T antigen antibody precipitated no eTBP from uninfected \(\alpha_3\) cell extracts but it clearly precipitated eTBP from extracts of SV40-infected \(\alpha_3\) cells. The control immunoprecipitation of HeLa/SV40 extracts showed no eTBP as expected. A similar experi-
ment using anti-T antigen to immunoprecipitate a3 and a3/SV40 extracts showed that the expected results were obtained when the Western blot was probed with anti-TBP antibody (not shown).

Treatment of extracts with DNase followed by immunoprecipitation in the presence of DNase resulted in no alterations in the above data, which suggest that T antigen and eTBP communoprecipitated [not shown]. This indicates that communoprecipitation resulted from protein–protein interactions and not by tethering attributable to binding to a common piece of DNA.

**Coimmunoprecipitation of T antigen with anti-human TAF antibodies**

The above data suggest strongly that a stable complex exists in vivo containing large T antigen and TBP or TFIID. To determine whether large T antigen communoprecipitated with other components of TFIID we performed similar immunoprecipitations with monoclonal antibodies to three different human TAFs: hTAF$_{n_{100}}$ (analog of dTAF$_{n_{80}}$), hTAF$_{n_{130}}$ (analog of dTAF$_{n_{110}}$), and hTAF$_{n_{250}}$ (analog of dTAF$_{n_{250}}$). The a3 and a3/SV40 extracts were precipitated with the various antibodies and the precipitates were analyzed by Western blotting using anti-T antibody to probe the blots. Figure 8 shows that antibodies to each hTAF (Anti 100, Anti 130, and Anti 250) communoprecipitated large T antigen from a3/SV40 extracts in a fashion similar to the precipitation of T antigen with eTBP using anti-HA antibody. A control monoclonal antibody against the human hnRNPC1 protein showed no precipitation of T antigen. As in the other immunoprecipitation experiments, the coprecipitation of T antigen with anti-TAF antibodies was not altered when the extracts were treated with DNase and the immunoprecipitations were done in the presence of DNase (data not shown). In addition, Western blot analysis has shown that the anti-TAF antibodies do not cross-react with T antigen [not shown]. The overall data indicate that large T antigen communoprecipitates because it is in complex with TFIID.

**Large T antigen purifies as a complex with TFIID**

Nuclear extract from SV40-infected a3 cells was fractionated on phosphocellulose to produce the TFIID fraction as described by Dignam et al. [1983b] [see Materials and methods]. Figure 9A shows a Western blot of the 1.0 M KCl eluate that contains TFIID. The same blot appears in each lane, it has been probed consecutively, first with anti-large T antigen (lane 1), then with anti-HA to detect the eTBP (lane 2), then with anti-hTAF$_{n_{130}}$ (lane 3), and finally with anti-hTAF$_{n_{250}}$. To retain bound proteins the blot was not stripped between probings. T antigen and each component of TFIID were detected readily in the 1 M fraction. Thus, in agreement with the data suggesting an association between T antigen and TFIID, these data indicate that some large T antigen copurifies with TFIID.

In Figure 9B we performed immunoprecipitation experiments to confirm that the copurification of large T antigen with TFIID was attributable to an interaction between large T antigen and TFIID. The purified TFIID fraction was immunoprecipitated with either anti-HA antibody [aHA], to precipitate the eTBP and holOTFIID, or a control antibody, anti-hnRNPC1 antibody [aC1]. The precipitates were subjected to Western blot analysis and probed with anti-large T antigen antibody. Figure 9B clearly shows that large T antigen immunoprecipitated suggesting that copurification over phosphocellulose results from a physical interaction with TFIID.

**Trans-activation mutants of large T antigen that fail to rescue the ts defect in TAF$_{n_{250}}$ also fail to interact with TFIID**

In Figure 3A,B we showed that inA2815 was the large T antigen mutant most defective in its ability to rescue the ts defect in TAF$_{n_{250}}$. This correlated with a severe defect in trans-activation of the SV40 late promoter [Zhu et al. 1991]. If these functions of large T antigen are mediated through a complex with TFIID then it would be predicted that a mutant like inA2815 may be unable to form such complexes. To test this we transfected ts13 cells at both 32°C and 39°C with plasmids expressing wild-type large T antigen, inA2815 and inA2817 [which
Figure 9. Large T antigen copurifies in complex with TFIID. Nuclear extract from SV40-infected a3 cells was fractionated on phosphocellulose (see Materials and methods). [A] The 1.0 M eluate containing TFIID was analyzed by Western blotting. The same blot appears in each lane [1-4] and has been probed consecutively, with anti-large T antigen [lane 1], anti-HA to detect the eTBP [lane 2], anti-hTAF$_{130}$ [lane 3], and anti-hTAF$_{250}$. To retain bound proteins the blot was not stripped between probings. [B] The same 1.0 M fraction was immunoprecipitated anti-HA antibody (aHA) or a control antibody, anti-hnRNPC1 antibody (aC1). The precipitates were subjected to Western blot analysis and probed with anti-large T antigen antibody.

Figure 10. Coimmunoprecipitation of large T antigen and TBP from transfected ts13 cells. The ts13 cell line was transfected at 32°C and 39°C with 5 μg of plasmid encoding either wild-type large T antigen [p6-1AL] or mutants inA2815 or inA2817. Forty-eight hr after transfection nuclear extracts were prepared and immunoprecipitated using anti-TBP antibody. The precipitates were analyzed by Western blotting. The input lanes show the large T antigen detected in 20 μg of the crude nuclear extracts prepared at 32°C or 10 μg of the crude nuclear extracts prepared at 39°C. The αTBP precipitate [Ppt.] lanes show the T antigen coimmunoprecipitated using anti-TBP antibody with 400 μg of the crude nuclear extracts prepared at 32°C or 200 μg of the crude nuclear extracts prepared at 39°C.

Discussion

A TAF-like function for large T antigen

Our previous studies (Gruda and Alwine 1991; Gilinger and Alwine 1993, Gruda et al. 1993) and studies of others (Casaz et al. 1991, 1995; Kelly and Wildeman 1991; Rice and Cole 1993) have suggested that transcriptional activation by SV40 large T antigen requires protein–protein
interactions between T antigen and both the basal transcription complex and upstream bound transcription factors. These data indicated that although T antigen interacts with the basal transcription complex it could not activate a promoter containing only a TATA element; activation was dependent on the additional interaction with the upstream bound factor. Such requirements for activation are similar to those of the TAFs that, as integral components of TFIIID, cannot mediate transcriptional activation unless they interact with upstream bound factors (Hoey et al. 1993; Chen et al. 1994). This similarity in mechanisms raised the question of whether large T antigen may perform a TAF-like function.

If large T antigen performs as a TAF it would be predicted that both genetic and biochemical evidence could be generated to show that large T antigen is associated with TFIIID and affects its function. In the present studies we have provided evidence showing that large T antigen can rescue the temperature-sensitive defect in TAF$_{250}$ in ts13 cells and that mutants in transcriptional activation cannot mediate rescue. This result suggests that large T antigen can function like a component of TFIIID by augmenting, or replacing, a function of TAF$_{250}$. In addition, it correlates this effect with a known transcriptional activation function of large T antigen.

That large T antigen is a component of TFIIID was indicated by in vitro binding studies and communoprecipitation experiments. The in vitro binding studies indicated that large T antigen interacts significantly with dTAF$_{150}$, dTAF$_{110}$, and dTAF$_{40}$, as well as hTAF$_{130}$, and hTAF$_{110}$ (data not shown). These data compliment our previous binding studies that indicated an interaction with TBP (Gruda et al. 1993). Hence large T antigen is able to interact with at least four components of TFIIID. The in vivo significance of interactions with multiple components of TFIIID was demonstrated by the finding that large T antigen in SV40-infected cell extracts was communoprecipitated using antibodies specific for cTBP, TBP, hTAF$_{130}$, and hTAF$_{250}$, under conditions where holo-TFIIID would be precipitated. In addition, we demonstrate that wild-type T antigen can be coprecipitated with TBP from ts13 cell extracts and that mutants that fail to rescue the ts defect in TAF$_{250}$ fail to coprecipitate. Finally, we have provided data showing that large T antigen purifies as a component of TFIIID. The combined data strongly suggest a TAF-like function where large T antigen is closely associated with TFIIID to mediate transcriptional activation and rescue.

**Mutants of large T antigen affecting trans-activation also affect rescue of the ts defect of TAF$_{250}$**

The examination of large T antigen mutants indicates that interaction with either p53 or Rb [and Rb-related family members] is not essential for the rescue of ts TAF$_{250}$. This is in agreement with other studies of transcriptional activation by large T antigen (Trifillis et al. 1990; Zhu et al. 1991). These findings indicate that transcriptional activation mechanisms mediated by these tumor suppressor proteins are not associated with the ability of large T antigen to rescue ts TAF$_{250}$. However, characterized trans-activation mutants inA2835 and inA2815 (Table 1) were found to be defective in rescue. Our data indicate that this defect results from the inability of the mutant large T antigen to complex with TFIIID. The mutation in inA2835 maps to the amino terminus. This correlates with both our in vitro binding data that indicated that dTAF$_{110}$ interacts with the amino-terminal 172 amino acids of T antigen and our previous data that indicated that TBP interacts within this same region (Gruda et al. 1993). Thus the amino-terminal mutation may be defective because of a loss of the ability to bind to TAF$_{110}$ or TBP. The mutation in inA2815 maps to the DNA-binding domain, however, it is unlikely that the loss of ability to bind to DNA is the cause of defects in trans-activation or rescue. First, the mutation that affects mutant inA2835 does not affect the DNA-binding domain. In addition, several previous studies have demonstrated that specific and nonspecific DNA binding by large T antigen is not essential for transcriptional activation (Keller and Alwine 1985; Gallo et al. 1988, 1990; Beard and Brugmann 1989; Zhu et al. 1991; Casaz et al. 1995). It has been proposed (Casaz et al. 1995) that sequences within the DNA-binding domain are also critical for correct protein folding. Thus mutations in this region may result in an aberrantly folded molecule that is unable to trans-activate. This suggests that the structure of large T antigen necessary for optimal binding of TAFs, and related proteins, may be formed from separate domains of large T antigen brought together only in the case of the full-length wild-type protein. Our observation that a GST fusion of full-length large T antigen was the only GST substrate that bound dTAF$_{150}$ and dTAF$_{40}$ agrees with this conclusion.

**Contributions of small t antigen**

We have tested the effects of small t antigen in transcriptional activation of the cycA promoter in ts13 cells at the nonpermissive temperature. Using this hamster cell line we observed that small t antigen could not rescue the ts defect in TAF$_{250}$. However, the presence of small t antigen with large T antigen increased synergistically the effects of large T antigen. Previously, small t antigen has been shown to transcriptionally activate a subset of promoters activated by large T antigen (Locken et al. 1988; Locken 1992). We have noted in the in vitro binding studies that small t antigen does interact with dTAF$_{110}$, an interaction that may relate to the synergy with large T antigen. However, it has been shown that small t antigen binds to protein phosphatase type 2A (PP2A) and inhibits its ability to dephosphorylate a variety of phosphoproteins (Pallas et al. 1990; Scheidtmann et al. 1991; Ruediger et al. 1992; Sontag et al. 1993). This has been shown to affect the activity of several transcription factors (Frost et al. 1994; Wheat et al. 1994). Hence, the synergy caused by small t antigen may relate to its effects on the phosphorylation state of transcription fac-
tors, maintaining them in a phosphorylated state that is better used by large T antigen for transcriptional activation.

Comparison of trans-activation by large T antigen and adenovirus Ela

Like large T antigen, adenovirus Ela is a promiscuous activator of many viral and cellular promoters and has been shown to interact with TBP and TFIIID (Boyer and Berk 1993; Geisberg et al. 1994). However, previously it has been proposed that large T antigen and Ela use different mechanisms for transcriptional activation (Loeken et al. 1986; Gruda et al. 1993). In the present data we have shown that the ability of large T antigen to rescue the ts defect in TAF$_{250}$ was not shared by Ela, again suggesting that these two viral activators use different mechanisms for the activation of transcription. One striking difference between Ela and large T antigen comes from a comparison of their abilities to activate transcription as Gal4 fusion proteins. Ela contains an activating region that functions well as a Gal4 fusion protein; however, neither large T antigen nor portions of it (portions that normally activate transcription of test promoters), contain such activation regions and will not function as Gal4 fusion proteins (Gruda et al. 1993). It has been proposed that the cellular target of the activating region of the Ela is the basic transcriptional apparatus, most likely TFIIH (Martin et al. 1990; Boyer and Berk 1993; Liu and Green 1994). These data suggest that the interaction of Ela with the basal complex may be similar to that of an upstream bound transcription factor (activator protein) by providing an activation domain for interaction with the basal transcription complex, for example, TAFs. Conversely, our data suggests that the interaction of large T antigen with TFIIH provides a new, TAF-like surface with which the activation domains of many activator proteins may interact. It has been proposed that the transcriptional effect of eukaryotic activator proteins is to increase the assembly of the preinitiation complex (Lin and Green 1991). Hence Ela and upstream bound transcription factors would accomplish this by providing activation domains that interact with the basal transcription complex. However, large T antigen would accomplish this by increasing the ability of other protein's activation domains to interact with the basal transcription complex. This model explains the results shown in Figure 1. Ela activated the cycA promoter at the permissive temperature because it provided an activation domain. However, large T antigen failed to activate because it has no activation domain and its TAF-like function is redundant at the permissive temperature in these cells. At the nonpermissive temperature the ts defect in TAF$_{250}$ resulted in conditions where the activation domain of Ela no longer functioned; however, large T antigen activated transcription because its TAF-like function rescued the ts defect.

Model

The above data and discussion lead to a model where large T antigen forms a stable complex with TFIIID and functions in this complex as a TAF. It seems likely that this function of large T antigen would mediate transcriptional effects. However, large T antigen influences the cell in many ways, including affecting the cell cycle, hence the interaction with TFIIID may also alter other nuclear and cellular functions of TAFs. Large T antigen's interactions with TFIIID may be mediated by binding to one or more components of TFIIID, for example, we have shown interaction with TBP and several TAFs (dTAF$_{110}$, dTAF$_{110}$, and dTAF$_{250}$). TBP, dTAF$_{150}$, and dTAF$_{110}$ also interact with dTAF$_{250}$, the major scaffolding protein of TFIIID. This similarity of interactions further suggests that large T antigen may provide a function analogous to TAF$_{250}$.

Materials and methods

Plasmids

Plasmids pRSV–Tex, pRSV–t, and pRSV–Ela contain cDNA copies of SV40 large T, small t antigen, and adenovirus Ela cDNA, respectively, under the control of the Rous sarcoma virus long terminal repeat (Loeken et al. 1988). Plasmid pRSV3–BglII, the control plasmid for the above constructs was generated by removing the T antigen cDNA from pRSV–Tex by cleavage with BglII and religation of the vector (Gruda and Alwine 1991). Plasmid p6-1AL encoded the entire early coding region of SV40 under the control of the SV40 early promoter (Keller and Alwine 1985) and thus, is able to produce both large T and small t antigen. Like p6-1AL, pT2811 (Bentivoglio et al. 1992) and pTIRb– [same as pSG5–K1, Kaelin et al. 1990] each encode the entire early region of SV40, however, the large T antigens produced by each are mutant. The pT2811 large T antigen cannot bind p53 and the pTIRb– large T antigen cannot bind the Rb. The control plasmid for p6-1AL, pT2811, and pTIRb– was pL16HX, which contains only the early SV40 promoter. A number of plasmids encoding mutants of T antigen, which have been characterized for transcriptional activation (Zhu et al. 1991) were used in transfection studies in ts13 cells, these are listed in Table 1. Each mutation is in the context of the early region and therefore, the wild-type control plasmid was p6-1AL and the null control was pL16HX. The mutant large T antigens produced are all full or near full length and, similar to p6-1AL, each plasmid has the SV40 origin of replication mutated so that amplification cannot occur (Zhu et al. 1991).

Plasmid pCMV–TAF$_{250}$ expressed human TAF$_{250}$ from the human cytomegalovirus immediate early promoter (Wang and Tjian 1994). The reporter plasmids, pCyclin A, containing the human cyclin A promoter upstream of the luciferase gene and pcfos–CAT, containing the human c–fos promoter with the CAT gene have been described previously (Wang and Tjian 1994). The six plasmids encoding the Drosophila TAFs have been described previously (Hoey et al. 1993; Chen et al. 1994). They contain cDNAs for either Drosophila TAF$_{40}$, TAF$_{60}$, TAF$_{80}$, TAF$_{110}$, TAF$_{150}$, or TAF$_{250}$, all under the control of the bacteriophage T7 promoter (Hoey et al. 1993) for use in in vitro transcription and translation systems. The plasmid encoding Drosophila TAF$_{250}$ contains the carboxy-terminal 180 amino acids and produces a protein migrating at 180 kD. Human TAF encoding plasmids pTIRbTAF$_{150}$ and pTIRbTAF$_{110}$ were generously provided by Naoko Tanese (New York University, NY) and pBSKShTAF32 was kindly provided by Robert Tjian (University of California, Berkeley).
Plasmids encoding fusion proteins between the glutathione-
bindmg site of glutathione-S-transferase and large T antigens
(full-length and pieces) or small T antigen have been described
previously (Gruda et al. 1993).

Cell culture, transfections, and infections
The ts13 cell line (Talavera and Basilico 1977) was propagated
and maintained in Dulbecco’s minimal essential medium
(DMEM) supplemented with 10% fetal calf serum at 32°C in 5%
CO2. Cells (3x10^5) were seeded on 60-mm plates and grown
overnight. Monolayers at ~70%-80% confluency were trans-
fected with 7 |xg of DNA by the calcium phosphate precipitation
procedure as described by Gruda et al. [1993]. Two micrograms
of the cycA–luciferase reporter plasmid were used with varying
amounts of DNA used per transfection . After the addition of DNA,
cells were placed at the appropriate temperatures, either 32°C for permissive conditions or 39°C for non-
permissive conditions. Cells were harvested 42–46 hr after transfection. For chloramphenicol acetyltransferase (CAT) anal-
ysis, cells were processed as described previously (Gruda et al. 1993). For luciferase analysis, cells were processed using the
Luciferase Assay System (Promega) and procedures supplied by
the manufacturer. Mini-nuclear extracts from ts13 cells were
made according to the protocol by Lee et al. (1988). Cells were
transfected with either wild-type or mutant T antigens (Table 1)
and placed in the incubators at the respective temperatures.
Cells were harvested 48 hr after transfection. For the cells at
39°C, cells were scraped off the plates as well as pelleted from
the media in the plates. The two pellets were combined and
used in the mini-nuclear extract protocol.

HeLa cells were propagated and maintained in Iscove’s me-
dium supplemented with 5% fetal calf serum at 37°C in 5%
CO2. The o3 cell line, a HeLa line that constitutively produces
an influenza HA epitope-tagged TBP (Zhou et al. 1992), was
maintained in DMEM supplemented with 5% fetal calf and 200
|pg/ml G418 at 37°C in 5% CO2. For infection with SV40, 9x10^5
HeLa or o3 cells were plated on 100-mm plates and grown over-
night. The medium was then replaced with 1 ml of medium
containing SV40 [WT strain 776] at an m.o.i. of 20 pfu/cell and
incubated for 1 hr at 37°C with periodic rocking. Then fresh
medium [9 ml] was added to each plate and the cells were incu-
bated for 48 hr at 37°C. Infected and mock infected cells were
harvested and nuclear extracts prepared by the procedure of Dig-
am et al. [1983a]. The protein concentration of the extracts was
determined using the Bradford assay.

Protein binding assay
Drosophila TAFs (dTAFs) 40, 60, 80, 110, 150, and 250(180)
were synthesized using the coupled in vitro transcription and
translation system (Promega). The proteins were labeled with
[35S]methionine and normalized for incorporation using TCA
precipitable counts.

Expression and purification of GST fusion proteins were done
as described previously (Gruda et al. 1993). The amounts of GST
proteins used in the binding reactions were normalized for ex-
pression using the Bradford protein assay and visually by silver
staining of the gel.

All binding reactions were performed at 4°C with constant
mixing on a Nutator. Glutathione agarose beads were bound
with NETN + and then incubated for another hour in 1 ml of
NETN + containing 8% BSA to block nonspecific protein bind-
ing to the beads. TCA precipitable counts of in vitro translated
TAFs (5X10^4) were then added to the beads and incubated for
1 hr at 4°C. The beads were then washed five times with
NETN +, boiled in SDS sample buffer, and separated by elec-
rophoresis in 9% SDS–polyacrylamide gels. Proteins were vi-
ualized by fluorography using Amplify [Amersham] and data were
quantitated using a Molecular Dynamics PhosphorImager.

Coimmunoprecipitations and Western analyses
Five hundred micrograms of HeLa or o3 cell nuclear extracts
were used in each immunoprecipitation or coimmunoprecipitation
experiment. Antibodies used included anti-T antigen
monoclonal antibody Pab419, anti-HA antibody mAb12CA5
from Boehringer Mannheim; and anti-TBP, anti-hTAFi,100,
anti-hTAFi,150, anti-hTAFi,250 all from Santa Cruz Biotechnol-
ogy. The control hRNPC1 antibody was a gift from Gideon
Dreyfuss (University of Pennsylvania). Immunoprecipitations
were performed using the method of Zhou et al. [1992] except
that binding was performed in 0.1 M KCl buffer D. Nuclear
extract was allowed to incubate with the respective antibody (1
|ug) and 10 |l of 50% vol/vol protein A-Sepharose beads (Phar-
macia) for 8 hr at 4°C with constant mixing on a Nutator.

Detection and quantification of the precipitates were TCA
precipitated using 5 |xg of BSA as carrier protein, and
then boiled in SDS-PAGE loading buffer. The eluted
proteins were then separated by electrophoresis on a 12% SDS–
polyacrylamide gel and transferred to nitrocellulose. Specific
proteins were detected by incubation with the appropriate anti-
body (either the anti-T antigen, anti-HA or anti-TBP antibod-
ies followed by visualization using the ECL luminescence kit;
Amersham).

For coimmunoprecipitations from ts13 cells at 32°C and
39°C, the same procedure was used except in this case only 100
|ug of nuclear extract was used in each coimmunoprecipitation
reaction, and the immunoprecipitates were washed with 0.1 M
KCl buffer D four times, followed by two washes with RIPA
buffer.

Phosphocellulose purification of TFIID
Nuclear extract from SV40-infected o3 cells was prepared as
described above and the nuclear extract was loaded onto a pre-
equilibrated phosphocellulose column at a concentration of 15
mg of nuclear extract per milliliter of column volume. Chromatography was performed as described by Dignam et al.
[1983b]. The 1.0 M KCl fractions collected from the column
were TCA precipitated using 5 |g of BSA as carrier protein, and
the precipitates were separated on an 8% SDS-PAGE gel, which
was then transferred to nitrocellulose. The resulting Western
blot was then probed for the presence of SV40 large T antigen,
cTBP, hTAFi,150, and hTAFi,250 sequentially without stripping
between probing.

To coimmunoprecipitate TBP and SV40 large T antigen from
the 1.0 M KCl fractions, the fractions were first dialyzed into 0.1
M KCl buffer D and then coimmunoprecipitations were per-
formed on each fraction using 2 |ug of anti-HA antibody and 50
|ug of 50% slurry of protein A beads in PBS. The complexes were
allowed to incubate for 8 hr and were washed three times with
0.1 M KCl buffer D plus 0.1% NP40, twice with 0.5 M KCl buffer
D and twice with 0.7 M KCl buffer D. The beads were then
resuspended in SDS sample buffer and separated on an 8% SDS-
PAGE gel that was transferred to nitrocellulose. The resulting
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B Damania and J C Alwine

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