$TAF_{II}250$-dependent transcription of cyclin A is directed by ATF activator proteins

Edith H. Wang,[1,3] Sharleen Zou,[2] and Robert Tjian[2]

[1]Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington 98195-7280 USA;
[2]Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720 USA

A specific mutation in $TAF_{II}250$, the largest subunit of the transcription factor TFIID, disrupts cell growth control in the temperature-sensitive mutant hamster cell line ts13. Transcription from the cyclin A and D1 but not the c-*fos* and *myc* promoters is also dramatically reduced in ts13 cells at the nonpermissive temperature. These findings provide an intriguing link between TAF-mediated transcriptional regulation and cell cycle progression. Here we report the mapping of an enhancer element in the cyclin A promoter (TSRE) that responds to mutations in $TAF_{II}250$. An analysis of chimeric promoter constructs reveals that the cyclin A TSRE can confer $TAF_{II}250$ dependence to the core promoter of c-*fos*. In addition, reciprocal hybrid promoter constructs suggest that $TAF_{II}250$ also contributes to the transcriptional properties of the cyclin A core promoter. We have purified and identified cellular activators that specifically bind to the TSRE and mediate transcription in a $TAF_{II}250$-dependent manner. By micropeptide sequencing, we determined that TSRE-binding proteins include members of the activating transcription factor (ATF) family. These results suggest that the *ts13* mutation of $TAF_{II}250$ has compromised the ability of TFIID to mediate activation of transcription by specific enhancer factors such as ATF, as well as to perform certain core promoter functions. These defects in $TAF_{II}250$ apparently result in the down-regulation of key molecules, such as cyclin A, which may be responsible for the ts13 cell cycle arrest phenotype.

[*Key Words:* Transcription; $TAF_{II}250$; cyclin A; ATF]

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Understanding the control of cell growth at the molecular level is a central problem in cancer biology. Tumors arise from cells that have somehow bypassed the checkpoints that regulate cellular proliferation. Normal growing cells proceed through a well-defined cell cycle composed of four distinct stages, G_1, S, G_2, and M (Murray and Hunt 1993). The signals that determine whether cells proceed from one stage to the next are regulated, in part, by the cyclins that bind to and activate the cyclin-dependent kinases (cdks) needed to drive cells through this proliferative cycle (Norbury and Nurse 1992; Pines 1993). Although much is known about the signaling pathways that govern cell proliferation, little is known about how the expression of the key proteins in these pathways is regulated. The level of cyclin proteins has been shown to oscillate throughout the cell cycle. Moreover, the deregulated expression of cyclin A and D1 has been found in a number of human tumors (Brechot 1993; Hunter and Pines 1994). Therefore, the periodic expression of these genes is critical for maintaining normal growth control.

Gene expression studies have identified the transcription factor IID (TFIID) as a potential target for transcriptional regulation. The binding of TFIID to the core promoter is required to nucleate the assembly of a functional transcription initiation complex (Van Dyke et al. 1988; Buratowski et al. 1989; Roeder 1996). TFIID itself is a multisubunit complex consisting of the TATA binding protein (TBP) and at least eight TBP-associated factors (TAFs), ranging in size from ~10 to 250 kD (Dynlacht et al. 1991; Tanese et al. 1991; Kokubo et al. 1993). The subunits of TFIID have been shown, at least in vitro, to play an essential role in transducing the activation signal from upstream regulatory proteins to the basal machinery by directly interacting with transcriptional activators (for review, see Verrijzer and Tjian 1996 and references therein). Additional studies have indicated that subunits of TFIID also are involved in directing core promoter selectivity (Kaufmann and Smale 1994; Verrijzer et al. 1995).

During the process of cloning the components of human TFIID, we discovered that the largest subunit,

[3]**Corresponding author.**
E-MAIL ehwang@u.washington.edu; FAX (206) 685-3822.

$TAF_{II}250$, is identical to cell cycle gene 1 (*CCG1*), a gene able to complement the late G_1 arrest observed in the temperature-sensitive mutant hamster cell line ts13 (Sekiguchi et al. 1991; Hisatake et al. 1993; Ruppert et al. 1993). Further characterization of the ts13 cell line demonstrated that the cell cycle defect can be attributed to a single base pair change in the coding region of the $TAF_{II}250$/*CCG1* gene, which results in a glycine to aspartic acid amino acid substitution at position 690 of the hamster protein (Hayashida et al. 1994). These studies provided the first piece of genetic evidence that TAFs are functionally important in vivo. More recently, mutant strains of *Saccharomyces cerevisiae* containing temperature-sensitive alleles of $yTAF_{II}145$, the yeast homolog of human $TAF_{II}250$, have been shown to arrest in G_1 at the restrictive temperature, a phenotype analogous to that of ts13 cells (Walker et al. 1996). These findings represent an intriguing and unexpected convergence of two extensively studied fields of research—the function of TAFs in transcription and cell cycle regulation.

Despite having a mutation in the essential TFIID, ts13 cells do not exhibit a global defect in gene expression. mRNA synthesis from only a subset of protein encoding genes is altered at the nonpermissive temperature (Liu et al. 1985; Wang and Tjian 1994; Sekiguchi et al. 1996). Apparently, the ts13 mutation in $TAF_{II}250$ has not disrupted all of the functions of TFIID that are required for transcription in general. Moreover, the overexpression of wild-type human $TAF_{II}250$ not only complements the ts13 cell cycle block but also overcomes the transcriptional defect detected at the nonpermissive temperature (Wang and Tjian 1994). Based on these findings, our current model is that the inability of ts13 cells to progress through the G_1 phase of the cell cycle is due to a promoter-specific defect in $TAF_{II}250$ function that compromises the expression of select genes, in particular, those involved in cell-cycle progression.

In previous studies, we began to characterize the transcriptional properties of TFIID complexes containing the mutant form of $TAF_{II}250$ and found that transcription from the cyclin A but not the c-*fos* promoter is reduced dramatically when ts13 cells are shifted from the permissive (33.5°C) to the nonpermissive (39.5°C) temperature (Wang and Tjian 1994). To extend these studies, we have analyzed the activity of additional cellular promoters by transient transfection assays in ts13 cells under permissive and nonpermissive conditions. We report here that the transcriptional activity of the cyclin D1 but not the *myc* promoter is altered upon inactivation of the $TAF_{II}250$ subunit of TFIID at 39.5°C. Cyclin A was utilized as the model gene to further investigate the gene-specific functions of $TAF_{II}250$ in ts13 cells. We have identified a *cis*-acting temperature-sensitive responsive element (TSRE) that regulates cyclin A expression and have purified cellular proteins that function through this regulatory element in a temperature-dependent manner. Four of the isolated polypeptides were determined by micropeptide sequencing to belong to the activating transcription factor (ATF) family of regulatory proteins. Interestingly, the ability of the TSRE to respond to the mutation in $TAF_{II}250$ can be transferred to heterologous promoters such as the c-*fos* core element. Analysis of chimeric promoters also suggests that the cyclin A core promoter element contributes to the transcriptional phenotype of ts13 cells. These studies indicate that TSRE-mediated regulation of cyclin A expression as well as cyclin A core promoter function have been disrupted in the mutant cells. Therefore, the largest subunit of the TFIID complex appears to provide multiple functional roles in the expression of the cyclin A gene during the cell cycle.

Results

ts13 *mutation in* $TAF_{II}250$ *alters the promoter activity of a subset of genes*

We have shown previously that transcription from the cyclin A but not the c-*fos* promoter is temperature-sensitive in ts13 cells (Wang and Tjian 1994). To further investigate this gene-specific defect in mRNA synthesis, which we speculate is responsible for the ts13 cell cycle arrest observed at the nonpermissive temperature, we examined the transcriptional activity of additional cellular promoters expressing growth control genes in vivo. Cyclin D1, like cyclin A, is a regulatory protein required for progression through the G_1 phase of the proliferative cell cycle. *myc* represents an immediate early gene whose expression is induced in many cell types upon treatment with mitogens or growth factors, similar to c-*fos*. The transcriptional activity of promoters from the cyclin D1 and *myc*, as well as the cyclin A and c-*fos*, genes fused to various reporters was determined by transient transfections in ts13 cells at the permissive (33.5°C) and nonpermissive (39.5°C) temperatures. To control for any variations in transfection efficiency and nonspecific temperature effects, the cells were cotransfected with a plasmid containing the β-galactosidase gene expressed from the SV40 early promoter, which is insensitive to the mutation in ts13 cells (Wang and Tjian 1994). Forty to 48 hr post-transfection, the relative transcriptional activity of each test construct was determined relative to β-galactosidase activity. Similar to our earlier findings with cyclin A and c-*fos*, a dramatic reduction in expression was detected from the cyclin D1 but not the *myc* promoter when ts13 cells were shifted from 33.5°C to 39.5°C (Fig. 1A). To demonstrate that this promoter-selective decrease in activity is due to the mutation in $TAF_{II}250$, the same set of reporter constructs was transfected into a stable ts13 transformant, ts13R3, which constitutively expresses wild-type human $TAF_{II}250$ and no longer exhibits a late G_1 arrest at 39.5°C (Wang and Tjian 1994). In contrast to ts13 cells, similar levels of activity were detected from all promoter constructs at the two temperatures in ts13R3 cells (Fig. 1B). These results confirm and extend previous findings (Liu et al. 1985; Wang and Tjian 1994; Sekiguchi et al. 1996) that ts13 cells do not exhibit global defects in transcription mediated by RNA polymerase II (Pol II) but instead display a gene-specific defect in mRNA synthesis that can be attributed to the mutation in $TAF_{II}250$.

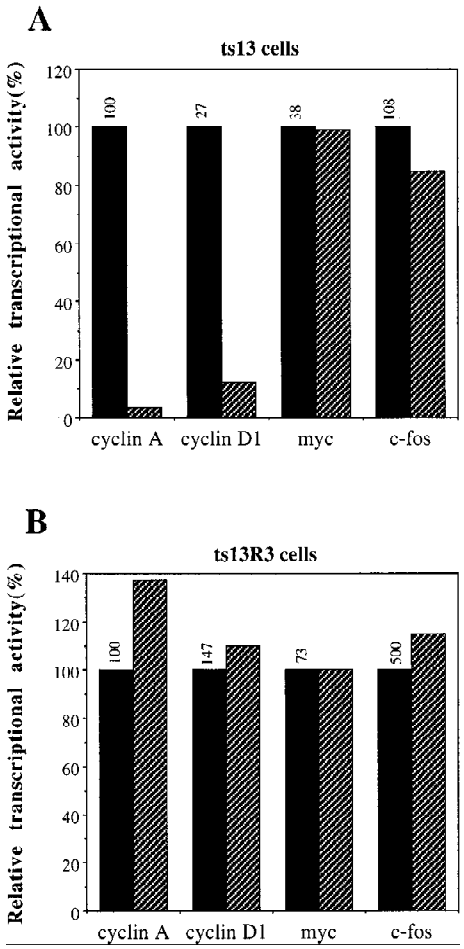

Figure 1. Transcriptional defect in ts13 cells is promoter specific. Reporter constructs containing the indicated cellular promoter upstream of the luciferase or chloramphenicol acetyltransferase (CAT) gene were cotransfected with a β-galactosidase expression vector into ts13 (*A*) and ts13R3 (*B*) cells maintained at 33.5°C (solid bars) or 39.5°C (hatched bars). Between 40 and 48 hr post-transfection, luciferase or CAT activity was measured and normalized for β-galactosidase activity. The transcriptional activity of each promoter at 39.5°C is expressed as a percentage of the activity detected at 33.5°C (given a value of 100%) in the same cell line. The relative activity of each promoter at 33.5°C with respect to cyclin A (given a value of 100) is also shown above each bar. The data represent results observed in three independent transfection experiments, each performed in duplicate.

Mapping the cis*-acting TSRE in the cyclin A promoter*

It still remains to be determined whether the decrease in cyclin A and D1 transcription at the nonpermissive temperature is a direct or indirect consequence of the cells arresting in late G_1. To address this issue, we set out to identify and characterize the regulatory factors involved in mediating cyclin A transcription. The human cyclin A promoter resides in an ~500 bp fragment and contains potential binding sites for a number of sequence-specific transcriptional regulators including Sp1, ATF, p53, E2F, NF-1, and AP-1 (Henglein et al. 1994; Yamamoto et al. 1994). However, it is expected that only a subset of these activators will respond to the $TAF_{II}250$ mutation and display a transcriptional defect in ts13 cells. As a first step toward identifying the specific activators that might be dependent on $TAF_{II}250$ function, we carried out mutagenesis studies of the cyclin A promoter to map the *cis*-acting element(s) responsible for the temperature-sensitive expression of cyclin A observed in ts13 cells. A series of 5′ and 3′ deletion constructs driving luciferase expression was generated, and the relative transcriptional activity of these mutants was determined by transient transfections into ts13 and ts13R3 cells at the permissive and nonpermissive temperatures (Fig. 2). For each construct, the ratio of the activities detected at 33.5°C and 39.5°C was calculated. These experiments revealed that the wild-type construct (A-WT) was ~100 times more active at 33.5°C than at 39.5°C. Removal of the GC boxes (AΔ1) had a very modest effect on the level of expression and no significant influence on the temperature sensitivity of the cyclin A promoter. However, a larger deletion in which the putative ATF-binding site was removed (AΔ2) resulted in a promoter construct that was severely compromised, suggesting that this factor binding site is important for cyclin A expression, in agreement with previous studies carried out in human cells (Henglein et al. 1994; Desdouets et al. 1995; Yoshizumi et al. 1995). In fact, a 70-bp region encompassing the ATF site (AΔ5) was not only active compared to the wild-type promoter but also remained temperature-sensitive in ts13 cells, such that a 66-fold difference in activity was detected at 33.5°C and 39.5°C. More important, only a modest (two- to fourfold) difference in promoter activity was detected with any of these reporter constructs at the permissive and nonpermissive temperatures in ts13R3 cells (Fig. 2). Using this approach, we have defined an upstream transcriptionally active TSRE, ~24 bp in length and containing an ATF-binding site, responsible for the decrease in cyclin A gene expression in ts13 cells (Fig. 2).

The cyclin A TSRE can confer $TAF_{II}250$-dependence to a heterologous core promoter

Unlike cyclin A, transcription from the c-*fos* enhancer/promoter is not sensitive to the temperature-sensitive mutation in $TAF_{II}250$. It was therefore possible to test whether the TSRE enhancer identified in the cyclin A promoter can confer temperature-sensitive transcription to the core element of the c-*fos* promoter in ts13 cells. In particular, we wanted to determine whether the defect in the TFIID complex in ts13 cells is activator selective. To test this possibility, chimeric promoters were generated (Fig. 3) by fusing a region of the cyclin A enhancer containing the TSRE to the c-*fos* core promoter. The transcriptional activity of this hybrid construct was determined by transient transfection in ts13 and ts13R3 cells at the permissive and nonpermissive temperatures (Fig. 3). In contrast to the c-*fos* gene, a 30-fold decrease in promoter activity was observed from the cyclin A/c-*fos*

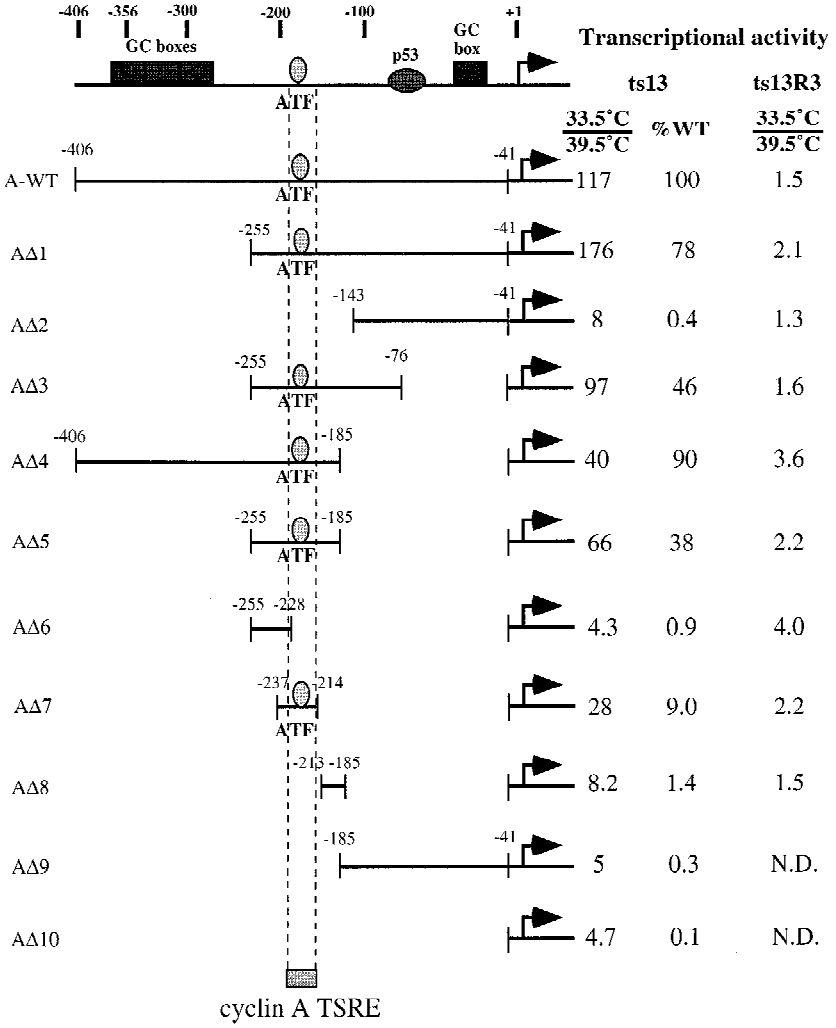

Figure 2. Upstream regulatory element in cyclin A exhibits temperature-sensitive transcriptional activity in ts13 cells. Deletion constructs of the cyclin A promoter cloned upstream of the luciferase gene were transfected into ts13 and ts13R3 cells as described in Materials and Methods. The transcriptional activity of each promoter at 33.5°C and 39.5°C was determined as a function of luciferase activity normalized for β-galactosidase activity. The ratios of activity detected at 33.5°C and 39.5°C are shown. The activity of each deletion construct relative to wild type (A-WT), given a value of 100%, in ts13 cells at 33.5°C is also shown. Indicated is the minimal cyclin A TSRE, which retains temperature-sensitive expression in ts13 but not in ts13R3 cells. The positions of potential protein binding sites including ATF are indicated. (N.D.) The experiment was not performed.

hybrid when ts13 cells were shifted from 33.5°C to 39.5°C. This result suggests that the TSRE can confer $TAF_{II}250$ dependence to heterologous core promoters. These findings indicate that specific enhancer/activator complexes are responsible for mediating cyclin A transcription in a $TAF_{II}250$-dependent manner, consistent with a coactivator role for the TFIID complex.

ATF-binding site displays temperature-sensitive transcriptional activity in ts13 cells

The ability of the cyclin A TSRE to confer temperature-sensitive transcriptional activity to a heterologous promoter prompted us to carry out the following genetic studies to determine whether the ATF site is responsible for the $TAF_{II}250$ requirement observed in vivo. We mutated sequences within or adjacent to the ATF site in the 24-bp cyclin A TSRE (see Fig. 5C, below). The wild-type and mutant elements were cloned upstream of a core promoter containing the TdT initiator, and the transcriptional activity of the resulting constructs determined by transient transfection into ts13 cells at 33.5°C and 39.5°C (Fig. 4). A double point mutation at the ATF site within the TSRE, which essentially abolished ATF binding, simultaneously produced a promoter element that was inactive in ts13 cells. In contrast, mutating sequences adjacent to the ATF site had no effect on the ability of the TSRE to activate transcription. Furthermore, this activity was temperature sensitive. These findings, taken together with our previous results, suggest that the transcriptional function of the ATF site in the TSRE is $TAF_{II}250$-dependent and required for high, regulated levels of cyclin A expression.

The cyclin A core promoter also contributes to temperature-sensitive transcription in ts13 cells

Although our studies with cyclin A/c-*fos* chimeric promoters establish a significant role for specific activators in the ts13 transcriptional defect, it was still possible that core promoter elements also contribute to the $TAF_{II}250$ requirement. In particular, it has become apparent that core promoters of different genes are not equivalent and that the interaction of TFIID with these

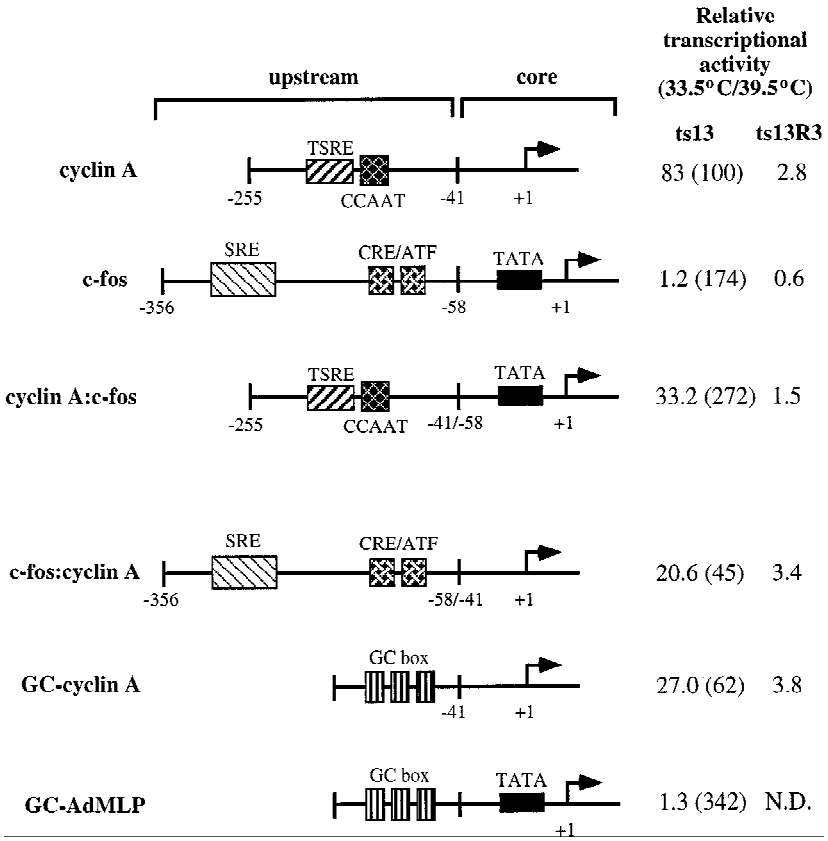

Figure 3. Transcriptional activity of chimeric cyclin A/c-*fos* promoters in ts13 cells. Promoter constructs were transiently transfected into ts13 and ts13R3 cells maintained at 33.5°C or 39.5°C. After 40–48 hr, the transcriptional activity of each promoter was determined and the ratio of activity detected at 33.5°C and 39.5°C shown. The relative activity of each promoter at 33.5°C is also given as a percentage of the activity detected for cyclin A (values shown in parentheses). A schematic diagram of the cyclin A, c-*fos*, and chimeric promoter upstream regulatory regions and core promoter elements are shown. The endpoints for the cyclin A core promoter are −41 to +5, and for the c-*fos* core promoter, −56 to +109. The position of the cyclin A TSRE, CCAAT-binding transcription factor site (CCAAT), serum response element (SRE), cAMP responsive element/activating transcription factor binding site (CRE/ATF), and GC-rich protein binding sites (GC box) are indicated. Not all known and potential protein binding sites are shown.

essential promoter elements may be important for gene-specific regulation (Hansen and Tjian 1995; Kaufmann et al. 1996). The core promoter of most RNA Pol II genes can be subdivided into at least two distinct elements: (1) the TATA box and (2) less well-defined elements that include the initiator as well as additional TAF binding sequences found downstream of the transcription start site (Smale 1994; Verrijzer et al. 1995). A comparison of the cyclin A and c-*fos* core promoter regions reveals distinct sequence elements. Most notably, the cyclin A promoter lacks a strong consensus TATA box, whereas c-*fos* contains a good TBP-binding site. To test the contribution, if any, of core promoter elements to the transcription defect in ts13 cells, we also generated chimeric templates by interchanging the core elements of the cyclin A and c-*fos* genes (Fig. 3). Interestingly, the hybrid promoter consisting of the c-*fos* enhancer region fused to the cyclin A core element displayed temperature sensitivity when transfected into ts13 cells. Because the wild-type c-*fos* promoter is insensitive to the *ts13* mutation in $TAF_{II}250$, these results suggest that not only does TSRE-mediated activation occur in a $TAF_{II}250$-dependent manner but that the transcriptional activity of the cyclin A core promoter element itself also may be $TAF_{II}250$ dependent.

However, we have not ruled out the possibility that the temperature-sensitive nature of the cyclin A core promoter when fused to the c-*fos* upstream element is

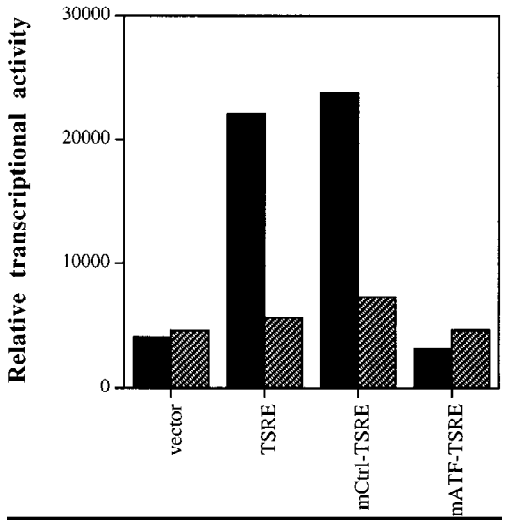

Figure 4. ATF binding site in the TSRE is temperature sensitive and important for cyclin A transcription. Reporter constructs containing either no TSRE (vector), wild-type (TSRE), or a mutant TSRE in which sequences adjacent to (mCtrl–TSRE) or within the ATF site (mATF–TSRE) have been changed upstream of the TdT initiator were transfected into ts13 cells maintained at either 33.5°C or 39.5°C. The relative transcriptional activity of each promoter was determined as described in Materials and Methods. The sequence of the wild-type and mutant DNA fragments is given in Fig. 5C.

merely a consequence of ATF sites already present in the c-*fos* upstream region (Sassone-Corsi et al. 1988; Hipskind and Nordheim 1991). Therefore, a promoter construct containing three copies of a GC-rich factor binding site (GC box) upstream of the cyclin A core promoter was generated and transfected into ts13 cells (Fig. 3). We observed that GC–cyclin A was also responsive to $TAF_{II}250$ in ts13 cells. However, when the GC boxes were cloned upstream of the adenovirus major late promoter (AdMLP) (Fig. 3) or E1B (data not shown) core promoter, comparable levels of transcriptional activity were detected from both constructs at the two temperatures. In earlier in vitro transcription studies, we had observed that the ability of the transcriptional activator Sp1, a GC box-binding protein, to stimulate transcription in ts13 nuclear extract is temperature sensitive (Wang and Tjian 1994). These data appear at first to contradict our finding that GC box-mediated transcription is unaltered by $TAF_{II}250$ inactivation in ts13 cells. Although GC boxes represent Sp1-binding sites, there actually exists a family of proteins that bind to GC-rich sequences. Therefore, it is possible that a transcriptional regulator besides Sp1, which does not functionally require $TAF_{II}250$, is interacting with the GC boxes in ts13 cells resulting in the temperature insensitivity of the GCAdMLP in ts13 cells. These studies collectively suggest that $TAF_{II}250$ is required for the function of a subset of upstream regulatory protein-binding sites as well as specific core promoter elements.

Identification of sequence-specific DNA-binding proteins that recognize the cyclin A TSRE

ATF is a family of transcription factors that includes both ATF and CREB (cAMP responsive element binding) proteins (for review, see Lee and Masson 1993). Currently, there are at least 10 members that belong to this large family of transcriptional regulators. They were all cloned based on their ability to recognize the DNA consensus sequence 5′-TGACGTCA-3′. Because of the complexity of this family of transcription factors, we took a biochemical approach to purify the protein(s) that interact with the cyclin A TSRE. The ability of ATF/CREB-binding sites to mediate differential transcription responses has been suggested to be determined by sequences flanking the consensus binding site. Such sequences have been shown to influence the DNA-binding specificity of distinct ATF/CREB family members. To identify nuclear proteins that interact specifically with the cyclin A TSRE, gel mobility shift assays were carried out using a labeled DNA probe encompassing the cyclin A TSRE and adjacent sequences. Because of the availability of many reagents generated against human ATF/CREB proteins, gel mobility shift assays were carried out in both ts13 hamster and HeLa human nuclear extracts. As shown in Figure 5A, similar DNA–protein complexes, based on their relative electrophoretic mobility, were detected in the two cell lines.

To determine whether these factors were binding specifically to the ATF site within the cyclin A TSRE, cold competitor DNA was added to the binding reactions carried out in HeLa extracts (Fig. 5B,C). We observed that the retarded DNA–protein complexes were competed away by the addition of increasing amounts of the wild-type TSRE (Fig. 5B, lanes 2–5) or a mutant in which sequences outside of the ATF site had been changed (Fig. 5B, lanes 6–9). However, a TSRE containing a mutant ATF site was unable to function as an efficient competitor (Fig. 5B, lanes 10–13). Identical results were obtained with DNA–protein complexes detected in nuclear extracts from ts13 cells (data not shown). These results demonstrate that both ts13 and HeLa cells contain nuclear factors that specifically recognize the ATF site in the cyclin A TSRE.

Purification of TSRE-binding proteins that mediate transcriptional activation through the cyclin A TSRE

To elucidate the $TAF_{II}250$-dependent pathway important for cyclin A gene expression, we set out to purify the factors that bind to the cyclin A TSRE by conventional ion exchange and DNA affinity chromatography. The fractionation scheme, outlined in Figures 6A and 7A, was developed to purify TSRE-binding activity from HeLa cells. Throughout the purification procedure, proteins eluted from each ion exchange column were monitored for TSRE-binding activity by gel mobility shift assays (Fig. 6B,C). Fractions containing the peak of DNA-binding activity were pooled, their ionic strength adjusted by dialysis, and the resulting proteins subjected to subsequent column chromatography. The last step in the purification scheme involved passing the Poros HS fractions over a sequence-specific DNA affinity column containing multiple copies of the cyclin A TSRE (Fig. 7A). The binding activity of interest was found to elute with 1.0 M KCl and consisted of ~10 distinct polypeptides as detected by silver staining of SDS–polyacrylamide gels (Fig. 7B).

To determine the identity of the different polypeptides, sufficient quantities of the cyclin A TSRE-binding proteins were purified from HeLa cells for micropeptide sequencing analysis. Approximately 3–5 μg of each polypeptide was isolated from 80 liters of HeLa cells. The sequences obtained revealed that four of the polypeptides present in our purified preparations are the transcriptional activators ATF1, CREB1, and ATFa2, members of the ATF/CREB family of regulatory proteins (see Fig. 7B).

The ability of the TSRE-binding proteins to stimulate transcription from a DNA template containing the cyclin A TSRE was examined in an in vitro transcription system (Fig. 7C). Transcription reactions were carried out in HeLa nuclear extracts depleted of TSRE-binding activity by DNA affinity chromatography as described previously (Hai et al. 1988). We observed that the addition of increasing amounts of the TSRE-binding proteins stimulated transcription from a DNA template containing the cyclin A TSRE (Fig. 7C, lanes 1–3). No increase was observed with the promoter construct lacking such an element (Fig. 7C, lanes 4–6).

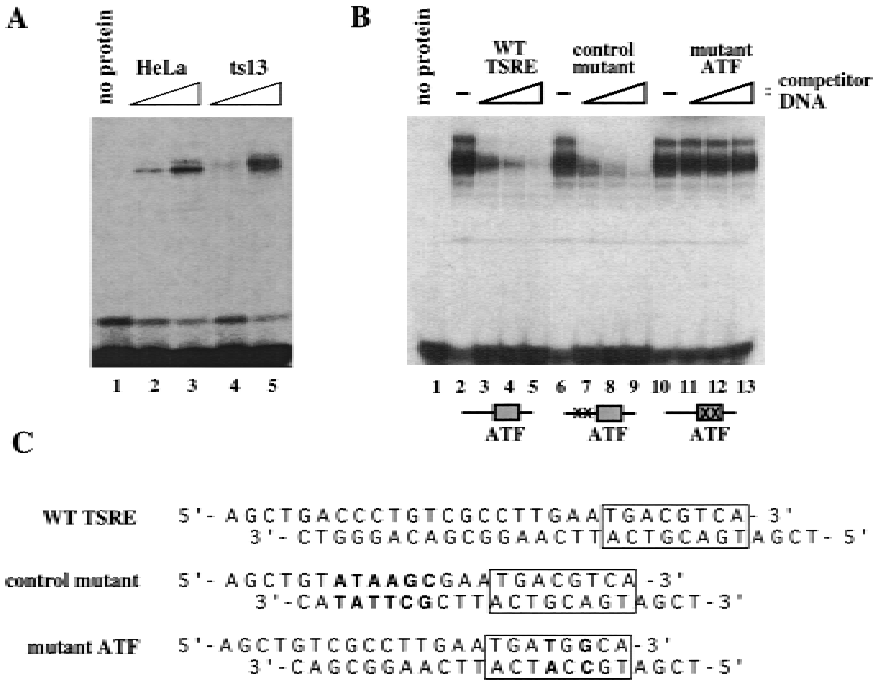

Figure 5. Identification of cellular proteins that bind sequence specifically to the cyclin A TSRE. (*A*) Similar DNA–protein complexes are detected in ts13 and HeLa nuclear extracts in gel mobility shift assays. No protein (lane *1*) or increasing amounts of HeLa (lanes *2*,*3*) or ts13 (lanes *4*,*5*) nuclear extracts were incubated with a ^{32}P-labeled DNA probe containing the cyclin A TSRE. After 1 hr incubation on ice, DNA–protein complexes were resolved on a 5% polyacrylamide gel and detected by autoradiography. (*B*) Multiple proteins specifically interact with the ATF site in the cyclin A TSRE. HeLa nuclear extracts were preincubated for 15 min on ice with increasing amounts of unlabeled wild-type TSRE (WT TSRE) (lanes *2–5*), control mutant TSRE (lanes *6–9*), or mutant ATF TSRE (lanes *10–13*) as competitor DNA followed by the addition of the labeled cyclin A TSRE DNA probe. The resulting DNA–protein complexes, after an additional 45 min incubation, were detected as described in *A*. A schematic representation of the competitor DNAs added to each reaction are shown below the lanes. (*C*) Sequence of wild-type and mutant TSRE competitor DNAs. The position of the ATF-binding site is indicated by the box. Base pair changes introduced into the mutant fragments are shown in boldface type.

Transcriptional activity of TSRE-binding proteins is functionally dependent on TAF$_{II}$250

Previously we had reported that Sp1- and VP16-mediated activation is temperature sensitive in ts13 nuclear extracts in vitro (Wang and Tjian 1994). To determine whether the transcriptional activity of the cyclin A TSRE-binding proteins also is dependent on wild-type TAF$_{II}$250, transcription assays were carried out at different reaction temperatures in nuclear extracts prepared from ts13 cells that contain the temperature-sensitive mutant form of TAF$_{II}$250 in the TFIID complex (Fig. 8A). Similar to Sp1 and VP16, at 20°C, we observed that the addition of the TSRE-binding proteins stimulated transcription from a DNA template containing the cyclin A TSRE. In contrast, no increase in the amount of correctly initiated RNA was observed when the reaction mixtures were incubated at 30°C. However, we did detect a set of transcripts at 30°C in ts13 nuclear extracts that were slightly longer and may arise as a result of defects in the core promoter recognition properties of TAF$_{II}$250 at the nonpermissive temperature. These reaction products do

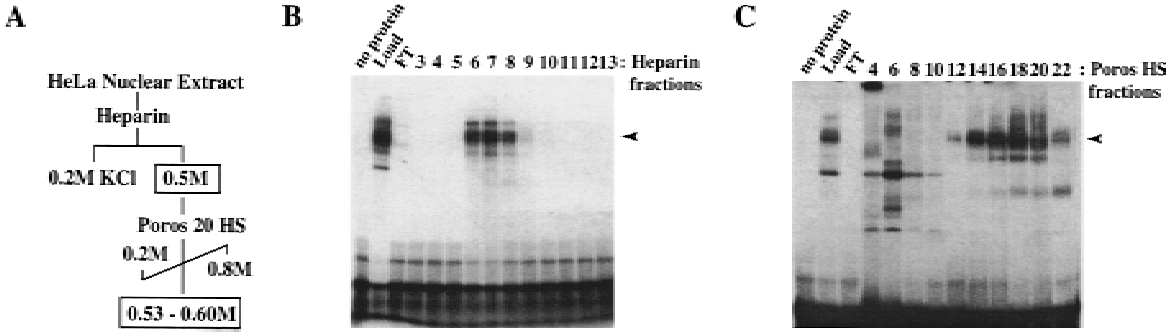

Figure 6. Purification of TSRE-binding proteins by ion exchange affinity chromatography. (*A*) Fractionation scheme used to isolate cyclin A TSRE-binding proteins from HeLa nuclear extracts. (*B*) Protein fractions eluted from the Heparin column and containing cyclin A TSRE-binding activity as determined by gel mobility shift assays. An amount of 0.5 µl of the indicated fractions was incubated with a ^{32}P-labeled cyclin A TSRE for 1 hr on ice. The binding reactions were subjected to 5% PAGE, and DNA–protein complexes visualized by autoradiography. The positions of DNA–protein complexes of interest are indicated by the arrowhead. (*C*) Elution profile of cyclin A TSRE-binding activity from Poros HS column. An aliquot (0.5–2 µl) of the indicated column fractions was assayed for TSRE-binding activity by gel mobility shift assays, as described in *B*. The peak of DNA-binding activity, indicated by the arrowhead, eluted between 0.53 and 0.6 M KCl.

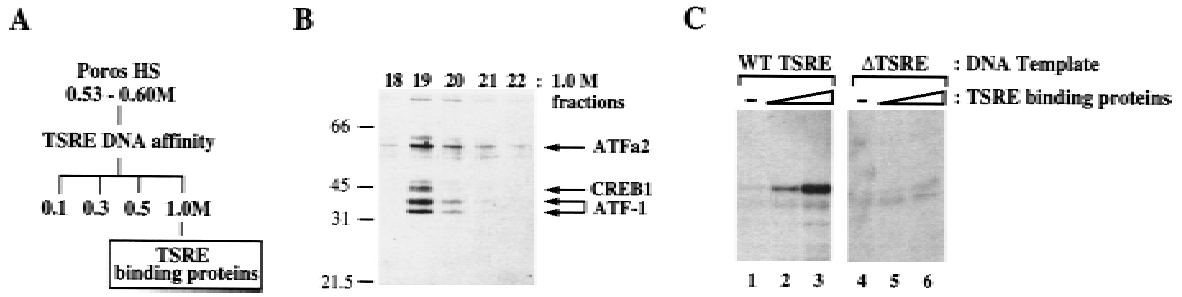

Figure 7. DNA affinity purification of TSRE-binding proteins. (*A*) Scheme for purifying TSRE-binding proteins from the Poros HS fractions using DNA affinity chromatography. (*B*) Silver-stained gel of cyclin A TSRE-binding proteins. Five microliters of the indicated protein fractions eluted from the DNA affinity column with 1.0 M KCl were separated on a 10% SDS–polyacrylamide gel and visualized by silver staining. Polypeptides identified by peptide sequencing are indicated. (*C*) TSRE-binding proteins mediate sequence-specific activated transcription in vitro. Increasing amounts of the TSRE-binding proteins, shown in *B*, were added to HeLa nuclear extracts depleted of TSRE-binding activity. Transcription from template DNA containing a wild-type (lanes *1–3*) or no (lanes *4–6*) cyclin A TSRE was detected by primer extension, 8% denaturing PAGE, and autoradiography.

not respond to the addition of the TSRE-binding proteins and most likely do not represent accurate initiation products from the test promoter. As expected, TSRE-mediated activation was detected at both temperatures when examined in ts13R3 (Fig. 8B) and HeLa nuclear extracts (Fig. 8C), each of which contain wild-type $TAF_{II}250$. It should be noted that although comparable levels of activation were detected at 20°C and 30°C in HeLa nuclear extracts, a noticeable reduction in TSRE-mediated transcription was observed in ts13R3 extracts. Albeit, ts13R3 cells no longer exhibit a growth arrest at 39.5°C, these cells still possess mutant, in addition to wild-type, $TAF_{II}250$. Therefore, the decrease in TSRE-dependent transcriptional activation in ts13R3 extracts at 30°C may be attributed to the inactivation of the mutant $TAF_{II}250$, present in TFIID complexes, that is fully functional at 20°C. To rule out the possibility that ts13 nuclear extracts are unable to support transcription at 30°C independent of activator, we tested the ability of the regulatory protein Gal4p53 to stimulate transcription in ts13 nuclear extracts. We found that the Gal4p53-mediated activation was temperature independent in ts13 extracts (data not shown). These findings suggest that the TSRE-binding proteins are unable to efficiently stimulate transcription in the mutant ts13 cells at the nonpermissive temperature and that the inactivation of the largest subunit of TFIID, $TAF_{II}250$, directly leads to the temperature-dependent decrease in cyclin A gene expression.

Discussion

Characterization of the mutant ts13 cell line by our laboratory and others has demonstrated that the transcriptional activity of only a subset of RNA polymerase II promoters is altered by a point mutation in $TAF_{II}250$, a component of the TFIID complex and transcriptional machinery. This finding suggests that $TAF_{II}250$ possesses a promoter-specific function(s) that has been compromised in the mutant ts13 cells. By deletion analysis, we have defined an upstream regulatory element (TSRE) in the cyclin A promoter region that is functionally dependent on $TAF_{II}250$. The construction of hybrid cyclin

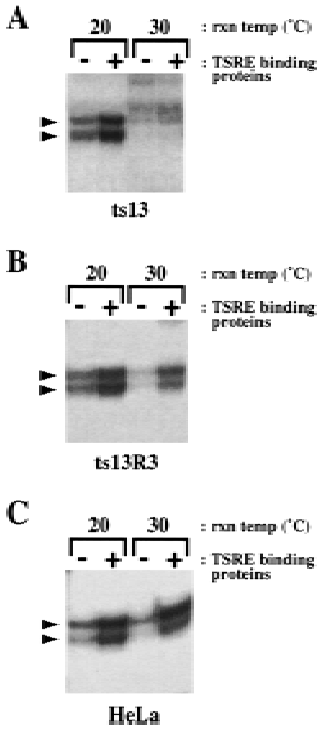

Figure 8. Transcriptional activity of TSRE-binding proteins is temperature sensitive in nuclear extracts containing mutant but not wild-type $TAF_{II}250$. In vitro transcription reactions were carried out in ts13 (*A*), ts13R3 (*B*), and HeLa (*C*) nuclear extracts either in the absence (−) or presence (+) of purified TSRE-binding proteins. After incubation at either 20°C or 30°C, the resulting reaction products were detected by primer extension followed by 8% denaturing PAGE and autoradiography. The reaction products, as a result of accurately initiated transcription from the TSRE–TICAT template DNA (see Materials and Methods for details), are indicated by the arrowheads.

A/c-*fos* promoters and genetic analysis confirmed that a specific element, the TSRE, is responsible for the activation of cyclin A transcription in a $TAF_{II}250$-dependent manner. In addition, our experiments indicate that the cyclin A core element also exhibits temperature-sensitive activity in ts13 cells. Therefore, the transcriptional activity of both the cyclin A TSRE and core promoter requires $TAF_{II}250$. In contrast, the entire c-*fos* regulatory region is insensitive to the ts13 mutation in the largest subunit of TFIID. These results suggest that the $TAF_{II}250$ mutation in ts13 cells not only affects the ability of some transcriptional activators to stimulate gene expression but also alters the activity of some core promoters.

Interestingly, the two $TAF_{II}250$-dependent transcriptional elements in the cyclin A promoter appear to be functionally independent units, each able to confer temperature-sensitive expression to heterologous promoter elements. This suggests that $TAF_{II}250$ carries out two separable functions (i.e., coactivator and core promoter recognition), both involved in the expression of the cyclin A gene.

Coactivator function of $TAF_{II}250$ in TSRE-mediated transcriptional activation

The activation of gene expression by transcriptional regulators in metazoans has been found to be largely dependent on the TAF components of TFIID (for review, see Verrijzer and Tjian 1996). These polypeptides are dispensable for basal transcription but essential for regulated transcription and were thus named coactivators (Pugh and Tjian 1990). Biochemical studies with recombinant proteins have identified specific interactions between individual TAFs and the activation domains of certain regulatory transcription factors. For example, an early report demonstrated that *Drosophila* (d)$TAF_{II}110$ binds directly to the glutamine-rich activation domains of the transcriptional activator Sp1 (Hoey et al. 1993). Mutational analysis revealed a correlation between loss of Sp1 activation and failure to bind to $dTAF_{II}110$ (Gill et al. 1994). More important, different classes of transcriptional activators specifically target distinct TAFs in the TFIID complex, and these protein–protein interactions have been shown to be required, at least in vitro, to transmit the activation signal from upstream regulatory proteins to the basal machinery (Chen et al. 1994; Sauer et al. 1996). In this report we have identified cellular activators that are functionally dependent on $TAF_{II}250$ and interact with a cyclin A transcriptional control element, the TSRE. The ability to reconstitute the temperature sensitivity of TSRE-mediated transcription in ts13 nuclear extracts in vitro strongly suggests that the decrease in cyclin A transcription is a direct result of the inactivation of $TAF_{II}250$ at the nonpermissive temperature. The inability of these proteins to stimulate transcription at 39.5°C in ts13 cells may be attributable to a defect in coactivator function. If $TAF_{II}250$ functions as a TSRE coactivator, one potential model is that the ts13 mutation hinders the ability of the TSRE-binding protein(s) to communicate with the transcription apparatus by disrupting a direct interaction between $TAF_{II}250$ and the TSRE-binding protein(s). In support of this hypothesis, we have carried out preliminary studies indicating that ATF-1, a polypeptide present in our TSRE protein preparations, binds directly to recombinant $TAF_{II}250$ in coimmunoprecipitation experiments (data not shown). However, our TSRE-binding activity consists of a mixture of polypeptides, and we have yet to determine which components are physiologically the most relevant for cyclin A TSRE activity.

Altered TFIID complexes in ts13 cells

$TAF_{II}250$ appears to be a core component of the TFIID complex and binds to a number of other subunits including TBP as well as several TAFs (Chen et al. 1994). Thus, the temperature-sensitive phenotype and defect in cyclin A transcription in ts13 cells may be accounted for by an alternative model in which the mutation in $TAF_{II}250$ has perturbed one or more of these $TAF_{II}250$–TAF interactions, thereby producing a TFIID complex containing substoichiometric amounts or even the complete absence of one or more of the TAFs. Such partial complexes, one would predict, would be unable to initiate high levels of transcription from a subset of RNA Pol II promoters that functionally require the missing subunit. For example, the absence of the TSRE coactivator could result in a significant decrease in cyclin A gene expression. It is possible that the mutant complexes still contain all of the core subunits of TFIID and appear perfectly intact. Under these circumstances, we speculate that the *ts13* mutation may have altered the nature of interactions between subunits, thereby disrupting the function of TFIID without disturbing its subunit composition. For example, $TAF_{II}250$ could affect the function of another component of TFIID by masking or unmasking this subunit via a temperature-dependent conformational change. Alternatively, the defect in ts13 cells may involve a function of the $TAF_{II}250$ protein itself. Additional experiments designed to distinguish between these different models will be required.

Requirement for $TAF_{II}250$ in core promoter function

A comparison of core elements and their activity in ts13 cells reveals that the c-*fos* and *myc* promoters contain TATA box sequences that are absent from the $TAF_{II}250$-dependent cyclin A and D1 promoters. TFIID is the primary basal transcription factor that possesses sequence-specific DNA-binding activity and selectively binds to TATA box sequences (Hoey et al. 1990; Peterson et al. 1990). The lack of a TATA box may necessitate a function of $TAF_{II}250$ not required at TATA-containing promoters. For example, $TAF_{II}250$ may be more directly involved in the recruitment of the TFIID complex to TATA-less core promoters, and the resulting DNA–protein complexes may require $TAF_{II}250$ to stabilize the preinitiation complex at the template.

Recently, studies have been reported in which tran-

scription in yeast bearing conditional alleles of $yTAF_{II}145$, the homolog of human $TAF_{II}250$ ($hTAF_{II}250$), was examined (Moqtaderi et al. 1996; Walker et al. 1996). Under conditions of $yTAF_{II}145$ depletion, the transcriptional activity of promoters containing suboptimal, nonconsensus TATA elements was reduced significantly (Moqtaderi et al. 1996). These findings are consistent with our results and indicate a preferential reduction in transcription from promoters containing weak or no TATA element upon inactivation of the $hTAF_{II}250/yTAF_{II}145$ subunit of TFIID. However, it seems unlikely that the mere presence or absence of a TATA box will solely be responsible for dictating the requirement for $TAF_{II}250$ at a particular enhancer/promoter. Our experiments with hybrid promoters indicate that both upstream enhancers (TSRE) and core elements play a role in mediating $TAF_{II}250$-dependent transcription.

Defect in cyclin A transcription arrests cells in late G_1?

Based on the transcriptional properties of ts13 cells, our current thinking is that the late G_1 block in the mutant cells is due to the down-regulation of select genes, such as cyclin A and D1, that are important for progression through the G_1 phase of the cell cycle. We do not believe that the ts13 growth defect is the result of a direct effect of $TAF_{II}250$ on cell cycle progression. Our hypothesis is consistent with previous findings that wild-type $TAF_{II}250$ overcomes both the temperature-dependent, gene-specific defect in transcription and cell cycle arrest observed in the mutant ts13 cells. Furthermore, mutations in other subunits of the TFIID, that is, $yTAF_{II}150$ (Walker et al. 1996) and $yTAF_{II}90$ (Apone et al. 1996), also have been shown to induce cell cycle defects in *S. cerevisiae,* suggesting that these components of TFIID are required for the proper transcription of additional growth control genes.

In summary, we have demonstrated that the regulated expression of cyclin A is dependent on the $TAF_{II}250$ subunit of TFIID. Specifically, $TAF_{II}250$ is involved in two regulatory pathways that control cyclin A transcription: (1) activation by the TSRE binding proteins, and (2) transcriptional activity of the cyclin A core promoter. Future studies of the regulatory components controlling cyclin A transcription will provide additional insight into the signal transduction pathway(s) that activates cyclin A expression during the proliferative cell cycle.

Materials and methods

Cell lines and cell culture

ts13 and ts13R3 cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone), and penicillin/streptomycin. ts13 cells were grown at 33.5°C, and ts13R3 cells at 39.5°C in a humidified incubator containing 10% CO_2. HeLa cells were cultured in Joklik's modified Eagle medium containing 5% calf serum and penicillin/streptomycin and grown in spinner culture at 37°C in the absence of CO_2.

Reporter plasmid constructs

The human cyclin A–luciferase construct (cycA–Luc) has been described (Yamamoto et al. 1994). Cyclin A promoter deletion plasmids AΔ1, AΔ2, AΔ4, AΔ5, and AΔ9 were generated by PCR. PCR products were sequenced and subcloned into *Hin*dIII–*Xho*I-digested cycA–Luc that lacks any promoter sequences (Δcyclin A). Mutant AΔ3 contains the filled-in *Alw*NI–*Hin*dIII fragment derived from the AΔ1 PCR product ligated into Δcyclin A. AΔ6, AΔ7, and AΔ8 were constructed by cloning double-stranded oligonucleotides containing *Hin*dIII and *Xho*I-compatible ends into Δcyclin A. The mouse c-*fos* promoter construct (p301–356 c-*fos*) has been described previously (Gilman et al. 1986). The cyclin D1 reporter plasmid was generated by cloning the filled-in *Pvu*II–*Eco*RI fragment of pD1–G0651 into the *Sma*I site of pGL2 basic (Promega). Chimeric promoters were constructed as follows. Cyclin A/c-*fos* hybrid was generated by ligating the filled-in *Hin*dIII–*Xho*I fragment of AΔ1 PCR product into the *Hin*dIII site of p301–56 c-*fos* (Gilman et al. 1986). The *Hin*cII–*Sma*I fragment of p301–356 c-*fos* was filled in and cloned into Δcyclin A with blunt ends to generate the c-*fos*/cyclin A chimera. GC–AdMLP contains three copies of human T-cell leukemia virus (HTLV)-box III in the *Sal*I site of TI-CAT (Pugh and Tjian 1990). GC–cyclin A was constructed by inserting the filled-in *Hin*dIII–*Xba*I fragment of BCAT-3 (Pascal and Tjian 1991), which contains three GC boxes, into Δcyclin A with blunt ends. The TSRE–TdT promoters were generated by cloning the filled-in double-stranded DNA fragments described in Figure 5C into the blunt-ended *Sal*I site of I-CAT (Pugh and Tjian 1990).

Transient transfection assays

ts13 and ts13R3 cells (2×10^5 to 5×10^5 cells/10-cm dish) were preincubated at 33.5°C or 39.5°C for 3–4 hr and subsequently were transfected with 1 μg of reporter plasmid, 1 μg of β-galactosidase expression vector, and 18 μg of sonicated salmon sperm DNA by calcium phosphate precipitation. The cells were maintained at either 33.5°C or 39.5°C and harvested between 40 and 48 hr post-transfection. β-Galactosidase activity was determined as described (Herbomel et al. 1984) and used to normalize for transfection efficiency. Luciferase activity was measured using the Promega luciferase assay system and CAT expression determined according to published procedures (Neumann et al. 1987).

Gel mobility shift assays

Reaction mixtures (20 μl) containing 10 mM HEPES (pH 7.6), 5 mM $MgCl_2$, 40 mM KCl, 0.1% NP-40, 25 μg/ml of poly[d(I-C)], and 4 pmoles of 37-bp ^{32}P-end-labeled double-stranded oligonucleotide representing the cyclin A TSRE were incubated with either a crude or partially fractionated HeLa nuclear extract for 1 hr on ice. DNA–protein complexes were resolved on 5% polyacrylamide gel in 0.25× TBE and visualized by autoradiography. The complementary oligonucleotides used for the DNA probe were 5′-AGCTGACCCTGTCGCCTTGAATGACGTCA-3′ and 5′- TCGATGACGTCATTCAAGGCGACAGGGTC-3′.

Purification of TSRE-binding proteins and peptide sequencing

HeLa nuclear extracts were prepared from 50–100 liters of cells (5×10^5 cells/ml) according to the procedure of Dignam et al.

(1983) and dialyzed against D buffer (20 mM HEPES at pH 7.6, 10% glycerol, 2 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT) containing 0.2 M KCl. The dialyzed extract was centrifuged at 25,000*g* to remove any precipitated material and subsequently loaded at 15 mg of protein/ml of resin onto a Heparin–agarose column equilibrated in D buffer containing 0.2 M KCl. The column was washed with three column volumes of D buffer containing 0.2 M KCl, and proteins eluted with threee column volumes of D buffer containing 0.5 M KCl. Fractions containing the peak of DNA binding activity, as determined by gel mobility shift assays, were pooled and dialyzed against HEMG (25 mM HEPES at pH 7.6, 10% glycerol, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT) containing 0.2 M KCl. Proteins were loaded at 25 mg of protein/ml of resin onto a Poros HS column equilibrated with HEMG containing 0.2 M KCl. The column was washed with 3–4 column volumes of 0.2 M KCl, and the bound proteins eluted with a linear gradient (10 column volumes) from 0.2 to 0.8 M KCl. Fractions containing TSRE-binding activity, as determined by gel mobility shift assays, were pooled, and NP-40 was added to a final concentration of 0.1%, dialyzed into HEMG containing 0.1 M KCl and subjected to DNA affinity chromatography. The DNA affinity column consisted of a 5′-biotinylated double-stranded oligonucleotide containing three copies of the cyclin A TSRE bound to streptavidin–agarose beads. The complementary oligonucleotides used were 5′-CTTGAATGACGTCAAGGT-GAATGACGTCAAGGTGAATGACGTCAAGG-3′ and 5′-CCTTGACGTCATTCACCTTGACGTCATTCACCTTGAC-GTCATTCAAG-3′. Approximately 0.5–1 mg of DNA was bound per milliliter resin. The TSRE-binding activity was preincubated for 30 min on ice with 20 μg/ml of poly[d(I-C)] and then loaded by gravity onto the DNA affinity resin equilibrated with HEMG containing 0.1 M KCl and 0.1% NP-40. After extensive washing with HEMG containing 0.1 M KCl and 0.1% NP-40, the retained proteins were eluted sequentially with 10 column volumes of HEMG containing 0.1% NP-40 and either 0.3, 0.5, or 1 M KCl. For peptide sequencing, the purified TSRE-binding proteins were TCA precipitated and resolved by SDS-PAGE. The proteins were transferred to nitrocellulose, and the membrane stained with Ponceau S. Individual polypeptides were excised and digested with trypsin. The eluted peptides were fractionated by reverse-phase HPLC and subjected to microsequencing.

In vitro transcription reactions

Transcription reactions (25 μl) containing 100 ng of template DNA, 10–50 μg of the indicated nuclear extract, 0–20 ng of TSRE-binding proteins, 10 mM HEPES (pH 7.6), 4 mM spermidine, 4 mM $MgCl_2$, 30 mM KCl, and 8 units of RNasin were incubated for 15 min at 20°C or 30°C followed by the addition of NTPs to a final concentration of 0.5 mM. After an additional 30–45 min incubation, reaction products were detected by primer extension, subjected to 8% denaturing PAGE, and visualized by autoradiography. Template DNAs included −237 to −214 of the cyclin A promoter cloned upstream of the TdT initiator region (7/8ICAT) and −255 to −185 of the cyclin A promoter fused to the AdMLP (TSRE–TICAT). Nuclear extracts from ts13, ts13R3, and HeLa cells were prepared according to published procedures (Dignam et al. 1983).

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References

Apone, L.M., C.M. Virbasius, J.C. Reese, and M.R. Green. 1996. Yeast $TAF_{II}90$ is required for cell-cycle progression through G_2/M but not for general transcription activation. *Genes & Dev.* **10:** 2368–2380.

Brechot, C. 1993. Oncogenic activation of cyclin A. *Curr. Opin. Genet. Dev.* **3:** 11–18.

Buratowski, S., S. Hahn, L. Guarente, and P.A. Sharp. 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* **56:** 549–561.

Chen, J.-L., L.D. Attardi, C.P. Verrijzer, K. Yokomori, and R. Tjian. 1994. Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* **79:** 93–105.

Desdouets, C., G. Matesic, C.A. Molina, N.S. Foulkes, P. Sassone-Corsi, C. Brechot, and J. Sobczak-Thepot. 1995. Cell cycle regulation of cyclin A gene expression by the cyclic AMP-responsive transcription factors CREB and CREM. *Mol. Cell. Biol.* **15:** 3301–3309.

Dignam, J.D., R.M. Lebovitz, and R.G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11:** 1475–1489.

Dynlacht, B.D., T. Hoey, and R. Tjian. 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **55:** 563–576.

Gill, G., E. Pascal, Z.H. Tseng, and R. Tjian. 1994. A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the $dTAF_{II}110$ component of the Drosophila TFIID complex and mediates transcriptional activation. *Proc. Natl. Acad. Sci.* **91:** 192–196.

Gilman, M.Z., R.N. Wilson, and R.A. Weinberg. 1986. Multiple protein-binding sites in the 5′-flanking region regulate c-fos expression. *Mol. Cell. Biol.* **6:** 4305-4316.

Hai, T., F. Liu, E. Allegretto, M. Karin, and M.R. Green. 1988. A family of immunologically related transcription factors that includes multiple forms of ATF and AP-1. *Genes & Dev.* **2:** 1216–1226.

Hansen, S.K. and R. Tjian. 1995. TAFs and TFIIA mediate differential utilization of the tandem *Adh* promoters. *Cell* **82:** 565–575.

Hayashida, T., T. Sekiguchi, E. Noguchi, H. Sunamoto, T. Ohba, and T. Nishimoto. 1994. The CCG1/$TAF_{II}250$ gene is mutated in thermosensitive G1 mutants of the BHK21 cell line derived from golden hamster. *Gene* **141:** 267–270.

Henglein, B., X. Chenivesse, J. Wang, D. Eick, and C. Brechot. 1994. Structure and cell cycle-regulated transcription of the human cyclin A gene. *Proc. Natl. Acad. Sci.* **91:** 5490–5494.

Herbomel, P., B. Bourachot, and M. Yaniv. 1984. Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. *Cell* **39:** 653–662.

Hipskind, R.A. and A. Nordheim. 1991. Functional dissection in vitro of the human c-fos promoter. *J. Biol. Chem.* **266:** 19583–19592.

Hisatake, K., S. Hasegawa, R. Takada, Y. Nakatani, M. Horikoshi, and R.G. Roeder. 1993. The p250 subunit of native TATA-binding factor TFIID is the cell-cycle regulatory protein CCG1. *Nature* **362:** 179–181.

Hoey, T., B.D. Dynlacht, M.G. Peterson, B.F. Pugh, and R. Tjian. 1990. Isolation and characterization of the Drosophila gene encoding the TATA box binding protein, TFIID. *Cell* **61:** 1179–1186.

Hoey, T., R.O.J. Weinzierl, G. Gill, J.-L.Chen, B.D. Dynlacht, and R. Tjian. 1993. Molecular cloning and functional analysis of drosophila $TAF_{II}110$ reveal properties expected of coactivators. *Cell* **72:** 247–270.

Hunter, T. and J. Pines. 1994. Cyclins and cancer II: Cyclin D and CDK inhibitors come of age. *Cell* **79:** 573–582.

Kaufmann, J. and S.T. Smale. 1994. Direct recognition of initiator elements by a component of the transcription factor IID complex. *Genes & Dev.* **8:** 821–829.

Kaufmann, J., C.P. Verrijzer, J. Shao, and S.T. Smale. 1996. CIF, an essential cofactor for TFIID-dependent initiator function. *Genes & Dev.* **10:** 873–886.

Kokubo, T., R. Takada, S. Yamashita, D.W. Gong, R.G. Roeder, M. Horikoshi, and Y. Nakatani. 1993. Identification of TFIID components required for transcriptional activation by upstream stimulatory factor. *J. Biol. Chem.* **268:** 17554–17558.

Lee, K.A. and N. Masson. 1993. Transcriptional regulation by CREB and its relatives. *Biochim. Biophys. Acta* **1174:** 221–233.

Liu, H.T., C.W. Gibson, R.R. Hirschhorn, S. Rittling, R. Baserga, and W.E. Mercer. 1985. Expression of thymidine kinase and dihydrofolate reductase genes in mammalian ts mutants of the cell cycle. *J. Biol. Chem.* **260:** 3269–3274.

Moqtaderi, Z., Y. Bai, D. Poon, A.P. Weil, and K. Struhl. 1996. TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* **383:** 188–191.

Murray, A. and T. Hunt. 1993. *The cell cycle.* W.H. Freeman and Company, New York, NY.

Neumann, J.R., C.A. Morency, and K.O. Russian. 1987. A novel rapid assay for chloramphenicol acetyltransferase gene expression. *BioTechniques* **5:** 444–447.

Norbury, C. and P. Nurse. 1992. Animal cell cycles and their control. *Annu. Rev. Biochem.* **61:** 441–470.

Pascal, E. and R. Tjian. 1991. Different activation domains of Sp1 govern formation of multimers and mediate transcriptional synergism. *Genes & Dev.* **5:** 1646–1656.

Peterson, M.G., N. Tanese, B.F. Pugh, and R. Tjian. 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. *Science* **248:** 1625–1630.

Pines, J. 1993. Cyclins and cyclin-dependent kinases: Take your partners. *Trends Biochem. Sci.* **18:** 195–197.

Pugh, B.F. and R. Tjian. 1990. Mechanism of transcriptional activation by Sp1: Evidence for co-activators. *Cell* **61:** 1187–1197.

Roeder, R. 1996. The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem. Sci.* **21:** 327–334.

Ruppert, S., E.H. Wang, and R. Tjian. 1993. Cloning and expression of human $TAF_{II}250$: A TBP-associated factor implicated in cell-cycle regulation. *Nature* **362:** 175–179.

Sassone-Corsi, P., J. Visvader, L. Ferland, P.L. Mellon, and I.M. Verma. 1988. Induction of proto-oncogene *fos* transcription through the adenylate cyclase pathway: Characterization of a cAMP-responsive element. *Genes & Dev.* **2:** 1529–1538.

Sauer, F., D.A. Wassarman, G.M. Rubin, and R. Tjian. 1996. TAF_{II}s mediate activation of transcription in the drosophila embryo. *Cell* **87:** 1271–1284.

Sekiguchi, T., Y. Nohiro, Y. Nakamura, N. Hisamoto, and T. Nishimoto. 1991. The human CCG1 gene, essential for progression of the G1 phase, encodes a 210-kilodalton nuclear DNA-binding protein. *Mol. Cell. Biol.* **11:** 3317–3325.

Sekiguchi, T., E. Noguchi, T. Hatashida, T. Nakshima, H. Toyoshima, T. Nishimoto, and T. Hunter. 1996. D-type cyclin expression is decreased and p21 and p27 CDK inhibitor expression is increased when tsBN462 CCG1/TAF_{II}250 mutant cells arrest in G1 at the restrictive temperature. *Genes Cells* **1:** 687–705.

Smale, S.T. 1994. Architecture of core promoters for eukaryotic protein-coding genes. In *Transcription: mechanisms and regulation* (ed. R.C. Conaway and J.W. Conaway), pp. 63–81. Raven Press, New York, NY.

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

Van Dyke, M.W., R.G. Roeder, and M. Sawadogo. 1988. Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. *Science* **241:** 1335–1338.

Verrijzer, C.P. and R. Tjian. 1996. TAFs mediate transcriptional activation and promoter selectivity. *Trends Biochem. Sci.* **21:** 338–341.

Verrijzer, C.P., J.-L. Chen, K. Yokomori, and R. Tjian. 1995. Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. *Cell* **81:** 1115–1128.

Walker, S.W., J.C. Reese, L.M. Apone, and M.R. Green. 1996. Transcription activation in cell lacking TAF_{II}s. *Nature* **383:** 185-188.

Wang, E.H. and R. Tjian. 1994. Promoter selective transcriptional defect in cell cycle mutant ts13 rescued by hTAF_{II}250 in vitro. *Science* **263:** 811–814.

Yamamoto, M., M. Yoshida, K. Ono, T. Fujita, N. Ohtani-Fujita, T. Sakai, and T. Nikaido. 1994. Effect of tumor suppressors on cell cycle-regulatory genes: RB suppresses $p34^{cdc2}$ expression and normal p53 suppresses cyclin A expression. *Exp. Cell Res.* **210:** 94–101.

Yoshizumi, M., C.-M. Hsieh, F. Zhou, J.-C. Tsai, C. Patterson, M.A. Perrella, and M.-E. Lee. 1995. The ATF site mediates downregulation of the cyclin A gene during contact inhibition in vascular endothelial cells. *Mol. Cell. Biol.* **15:** 3266–3272.