

The ts13 Mutation in the TAF_{II}250 Subunit (CCG1) of TFIID Directly Affects Transcription of D-Type Cyclin Genes in Cells Arrested in G₁ at the Nonpermissive Temperature

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The general transcription initiation factor TFIID contains the TATA-binding protein (TBP) and TBP-associated factors (TAFs) implicated in the function of gene-specific activators. Previous studies have indicated that a hamster cell line (ts13) with a point mutation in the TAF_{II}250/CCG1 (TAF_{II}250) gene shows temperature-sensitive expression of a subset of genes and arrests in late G₁ at 39.5°C. Here, we report the identification of cell cycle-specific (G₁-specific) genes that appear to be regulated directly through TAF_{II}250 both in vivo and in vitro. Transcription rates of several cell cycle-regulatory genes were determined by run-on assays in nuclei from ts13 cells grown at permissive (33°C) and nonpermissive (39.5°C) temperatures. Temperature-dependent differences in transcription rates were observed for cyclin A, D1, and D3 genes. In transient-transfection assays, the human cyclin D1 promoter fused to a luciferase reporter showed a temperature-dependent reduction in activity in ts13 cells but not in parental BHK cells. In in vitro assays, upstream sequence-dependent transcription from the human cyclin D1 promoter was significantly reduced in ts13 nuclear extracts preincubated at 30°C but not in similarly treated BHK nuclear extracts, and transcription in the ts13 extract was restored by addition of an affinity-purified human TFIID. Preincubation of the ts13 nuclear extracts did not affect the function of several GAL4-activation domain fusion proteins (GAL4-VP16, GAL4-p65, and GAL4-p53) on either the adenovirus major late or cyclin D1 core promoter bearing GAL4 sites, further indicating that the effect of the TAF_{II}250 mutation is both core promoter and activator specific.

TFIID is a general initiation factor which binds to core promoter elements of class II genes and nucleates the assembly of RNA polymerase II and other general initiation factors (TFIIA, TFIIB, TFIIE, TFIIIF, and TFIIH) into a functional preinitiation complex (reviewed in references 6 and 53). Consistent with its key role in transcription initiation, TFIID was also implicated as a target for gene-specific activators in the earliest studies of activation mechanisms. These studies showed both qualitative and quantitative effects of activators on TFIID binding that in turn correlated with enhanced recruitment and function of other general initiation factors (1, 23, 34, 71). Resolution of the polypeptide structure of TFIID, comprised of both a small TATA-binding polypeptide and a large number (>13 in human) of TATA-binding protein (TBP)-associated factors (TAFs) (reviewed in reference 6), has enhanced our understanding of the mechanisms involved. Various activators have been shown to interact directly with TBP or with specific TAFs (reviewed in references 6 and 67), altering either the binding of TFIID (56) or the conformation of the resulting promoter complex (23, 47). The significance of such interactions for activator-specific function has been indicated by a variety of approaches using mutated factors (17, 26, 28, 38) and partial TFIID complexes (9, 28, 56, 67).

TAF_{II}250 is the largest TAF identified and, by virtue of its ability to interact directly with TBP and with several other TAFs, has been suggested to play a key role in TFIID assembly (reviewed in reference 6). However, consistent with the possibility of a more direct role in activation, TAF_{II}250, like several other TAFs, has been found to interact directly both with

activators (7, 16, 60) and with another general initiation factor (6, 54). Perhaps most indicative of a regulatory function for TAF_{II}250, earlier studies (22, 55) identified human TAF_{II}250 as CCG1, a factor previously implicated as a cell cycle-regulatory protein (57, 58). A hamster cell line (ts13) carrying a point mutation in the CCG1/TAF_{II}250 gene shows temperature-sensitive cell growth and has been classified as a G₁ mutant (65). Following synchronization in the G₀/G₁ phase of the cell cycle at the permissive temperature (33°C), serum-stimulated ts13 cells arrest in late G₁ phase at the nonpermissive temperature (39.5°C [65]). Consistent with an earlier demonstration (36) that ts13 cells do not show a global defect in mRNA synthesis at the nonpermissive temperature, later studies have revealed that the temperature-sensitive mutation reduces mRNA levels of late G₁ or G₁/S boundary genes (e.g., genes encoding cyclin A, proliferating cell nuclear antigen, and DNA polymerase alpha) but not immediate-early G₁ genes (e.g., *c-fos*, *c-jun*, and *c-myc*) (29, 46).

Altogether, these results raise the possibility that the CCG1/TAF_{II}250 mutation may selectively affect transcription of a subset of genes whose products are involved in regulation of the G₁-to-S transition. Cell cycle-regulatory genes whose mRNA levels vary during the G₁-to-S transition are classified roughly into three classes (42). The first group includes the G₁ cyclins (C, D, and E) and their interacting cyclin-dependent kinases (cdks) (61), which together play key roles in signal processes involved in progression from G₁ to S phase. The second group includes transcription factors (e.g., c-Myb, E2F, p53, and the retinoblastoma protein [RB]) which confer cell cycle-regulated transcription on target genes and may be subject to regulation in late G₁/S phase (13, 20, 25, 68). The third group includes S-phase genes encoding enzymes involved in DNA metabolism (e.g., thymidine kinase, dihydrofolate reduc-

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tase, and DNA polymerase alpha) (27, 48) as well as regulatory factors such as cyclin A (51).

As a follow-up to these studies, the general objective of our work has been to identify specific genes whose transcription is regulated directly through TAF_{II}250 in a cell cycle stage-specific manner and then to elucidate the underlying mechanisms. Our initial approach has been to use both *in vivo* and *in vitro* assays to analyze transcription of G₁-specific cell cycle-regulatory genes as candidate target genes affected by the TAF_{II}250 mutation. Our results strongly suggest that the D-type cyclins (especially cyclin D1) are directly regulated through TAF_{II}250, and possible mechanisms for this regulation are discussed.

MATERIALS AND METHODS

Cell culture. The Syrian hamster cell line BHK-21 and the derived cell line ts13 were grown at 33°C in Dulbecco's minimal essential medium with 10% fetal bovine serum (FCS), penicillin (100 U/ml), and streptomycin (100 U/ml) (65). For synchronization by serum deprivation, cells were plated and grown at 33°C in medium containing 10% FCS for 24 h. Then medium was removed, and the cells were washed twice and incubated for 44 to 48 h in medium containing 0.25% FCS. For fluorescence-activated cell sorting analysis, cells were harvested and washed with 0.1% glucose in phosphate-buffered saline (PBS). Then cells were fixed in 50% ice-cold methanol and stained with 500 μ l of a solution of propidium iodide (50 μ g/ml) and RNase A (100 U/ml) in 0.1% glucose-PBS.

Determination of transcription rates. Nuclear run-on assays were performed with nuclei isolated from ts13 or BHK cells to determine relative rates of transcription (32). Cells grown in 150-mm-diameter dishes (1×10^6 to 1.2×10^6 cells) were used for each assay. DNA fragments corresponding to cDNAs of cyclin A (51), cyclin B (24, 52), cyclin C (33), cyclins D1, D2, and D3 (33, 41, 72), cyclin E (33), cdk2 (43), E2F1 (20, 25), RB (24), β -actin (50), and 5S RNA genes (30) were isolated and used as probes. Linearized pBluescript DNA was used as a negative control probe. Six micrograms of each alkaline-denatured (with 0.2 M NaOH) DNA preparation was immobilized on nitrocellulose filters in a slot blot apparatus (Schleicher & Schuell) and hybridized with [α -³²P]UTP-radiolabeled RNA. Typically 7.5×10^6 to 1.0×10^7 cpm (from fixed numbers of cells) were used for each hybridization in 1 \times TESS [20 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES; pH 7.4), 2 \times Denhardt's reagent, 400 mM NaCl, 2.6 mM EDTA, 0.07% sodium dodecyl sulfate, 0.2 mg of yeast RNA per ml] at 65°C for 16 h. Filters were washed sequentially with 2 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.5 \times SSC, at 65°C for 30 min in each case, and then treated with 2.5 μ g of RNase A per ml in 2 \times SSC at 37°C for 30 min. The levels of hybridized RNA were quantitated with a Molecular Dynamics PhosphorImager.

Transient-transfection assays. ts13 and BHK cells were plated at 5×10^5 to 8×10^5 cells per 100-mm-diameter dish and grown for 24 h at 33°C. Cells were transfected with calcium phosphate-precipitated DNA by a modification of the method of Chen and Okayama (8). Seven micrograms of plasmid DNA and 13 μ g of herring sperm DNA (Promega) as carrier were used for each transfection. After incubation with DNA for 24 h, cells were washed with PBS and allowed to grow for 24 h in medium containing 10% FCS. Then cells were treated with trypsin, divided among three 60-mm-diameter dishes, and synchronized by growth in medium containing 0.25% FCS for 2 days at 33°C (serum deprivation). Cells were released from serum deprivation by adding medium containing 10% FCS and allowed to grow for 16 h at either 33 or at 39.5°C. After preparation of cell extracts, luciferase assays were performed with the Promega luciferase assay system. Fluorescence was measured with a TROPIX ILA911 luminometer. The reporter genes used in these studies consisted of a series of 5'-deleted human cyclin D1 promoters fused with the luciferase gene (pXP2 derivatives) (21) and a simian virus 40 (SV40) promoter/enhancer fused to a luciferase gene (pSV2ALAD5') (70).

Preparation of nuclear extracts from hamster cells. Nuclear extracts were prepared by the method of Dignam et al. (12), with slight modification for smaller-scale preparations. Cells grown at 33°C were harvested from 80 to 100 dishes (150-mm diameter) and used for each nuclear extract preparation. Two different batches of nuclear extracts were mixed and used for *in vitro* transcription experiments.

In vitro transcription. *In vitro* transcription reaction mixtures (25 μ l) contained 60 mM KCl, 12% glycerol, 25 mM HEPES-KOH (pH 8.2), 7 mM MgCl₂, 1 mM dithiothreitol, 40 U of RNasin, 0.6 mM nucleoside triphosphates (ATP, GTP, CTP, and UTP), 50 to 70 μ g of nuclear extract (HeLa, BHK, or ts13), and 50 to 200 ng of DNA template; 2% polyvinyl alcohol was added to some of the experiments for cyclin D1 transcription in ts13 and BHK nuclear extracts. Plasmids containing cyclin D1 promoter regions fused with the luciferase gene (D1 Δ -973pXP2, D1 Δ -944pXP2, D1 Δ -742pXP2, D1 Δ -254pXP2, D1 Δ -78pXP2, and D1 Δ -29pXP2 [21]) and plasmids containing five GAL4 binding sites inserted upstream of the adenovirus major late (AdML) core promoter (5GAL4ML1 [32a]) or the human cyclin D1 core promoter (5GAL4D1 Δ -29) were used as templates. 5GAL4D1 Δ -29 was constructed from the DNA fragments derived

from D1 Δ -29pXP2 (-29 to +139) (21) and 5GAL4ML1. In some of the experiments, salmon testis DNA (250 μ g; Sigma) was incubated with nuclear extract for 20 min on ice before addition of the template to reduce the inhibition of specific transcription by nonspecific DNA binding protein interactions with template. Specific transcripts were detected by primer extension with specific primers (+45 to +68 for the cyclin D1 promoter and +72 to +92 for the AdML promoter) and mouse mammary tumor virus reverse transcriptase (Promega or GIBCO-BRL). The results were quantitated by PhosphorImager (Molecular Dynamics) analysis.

Expression and purification of recombinant proteins. Bacterially expressed FLAG-GAL4 fusion proteins were purified as described previously (10, 35) and consist of amino acids 1 to 94 of the GAL4 DNA binding domain fused to a duplicated copy of the transcriptional activation domain of p53 (amino acids 1 to 57), a single copy of the Sp1 transcriptional activation domain (amino acids 82 to 500), or a C-terminal portion (amino acids 416 to 550) of the largest subunit (p65) of NF- κ B. Vectors that express corresponding FLAG-tagged proteins were engineered by PCR methods (17a). These GAL4 fusion proteins were also reported to be functionally active *in vivo* and/or *in vitro* (4, 37, 66). Recombinant GAL4-VP16 protein was a generous gift from Dong-kun Lee.

RESULTS

Transcription rates of the cyclin D1 and D3 genes are selectively reduced at 39.5°C during G₁ phase in ts13 cells. To identify the target genes which are directly affected by the TAF_{II}250 mutation showing the CCG1 phenotype, the transcription rates of several cell cycle-regulatory genes that have functions in the G₁/S transition (cyclins A, B, C, D, and E, as well as cdk2, E2F, p53, RB, and TAF_{II}250 itself) were determined by run-on assays with nuclei isolated from ts13 cells grown at permissive (33°C) and nonpermissive (39.5°C) temperatures.

For these analyses, ts13 cells were first synchronized in the G₁/G₀ state by growth for 44 or 48 h at 33°C in the presence of 0.25% serum (serum deprivation). After readdition of 10% serum (serum addition), cell proliferation is restarted in cells grown at 33°C but not in cells grown at 39.5°C (Fig. 1A). Under these conditions, and using human cDNA probes, clear effects of temperature on nuclear run-on transcription rates were observed for cyclin A and D1 genes (Fig. 1B and C). For these two genes, transcription rates were as much as five to seven times higher at 33°C than at 39.5°C (Fig. 1C). The differential transcription rates of cyclin D1 were observed as early as 7.5 h after serum addition, whereas large differences in rates for cyclin A were observed only at 16 to 24 h after serum addition. No clear temperature-dependent differences in transcription rates were observed for other genes tested, including those encoding cdc2, p53 (data not shown), cyclin B, and TAF_{II}250. The transcription rate of the 5S gene, which is transcribed by RNA polymerase III, also was not affected by the ts13 mutation. These results indicate that the TAF_{II}250 mutation in ts13 cells selectively affects the transcription rates of specific genes (encoding cyclin A and cyclin D1) during the G₁ phase.

Given these results and the known functions of D-type cyclins in cell cycle progression, a more detailed analysis was undertaken with corresponding mouse cDNAs for three different types of D-type cyclins (D1, D2, and D3) as probes. As shown in Fig. 2, transcription patterns in synchronized serum-stimulated ts13 cells were significantly reduced at 39.5°C relative to 33°C for both cyclin D1 and D3 genes (Fig. 2A). However, some differences between the cyclin D1 and D3 transcription rates were observed. After cell growth for 7.5 h in the presence of serum, cyclin D1 transcription was induced fivefold at 33°C, whereas the uninduced level was maintained at 39.5°C. By contrast, during the same time period, the cyclin D3 transcription rate was modestly (50%) induced at 33°C but significantly (threefold) reduced at 39.5°C, leading to a differential effect of fivefold at the permissive temperature versus the nonpermissive temperature. The transcription signal for

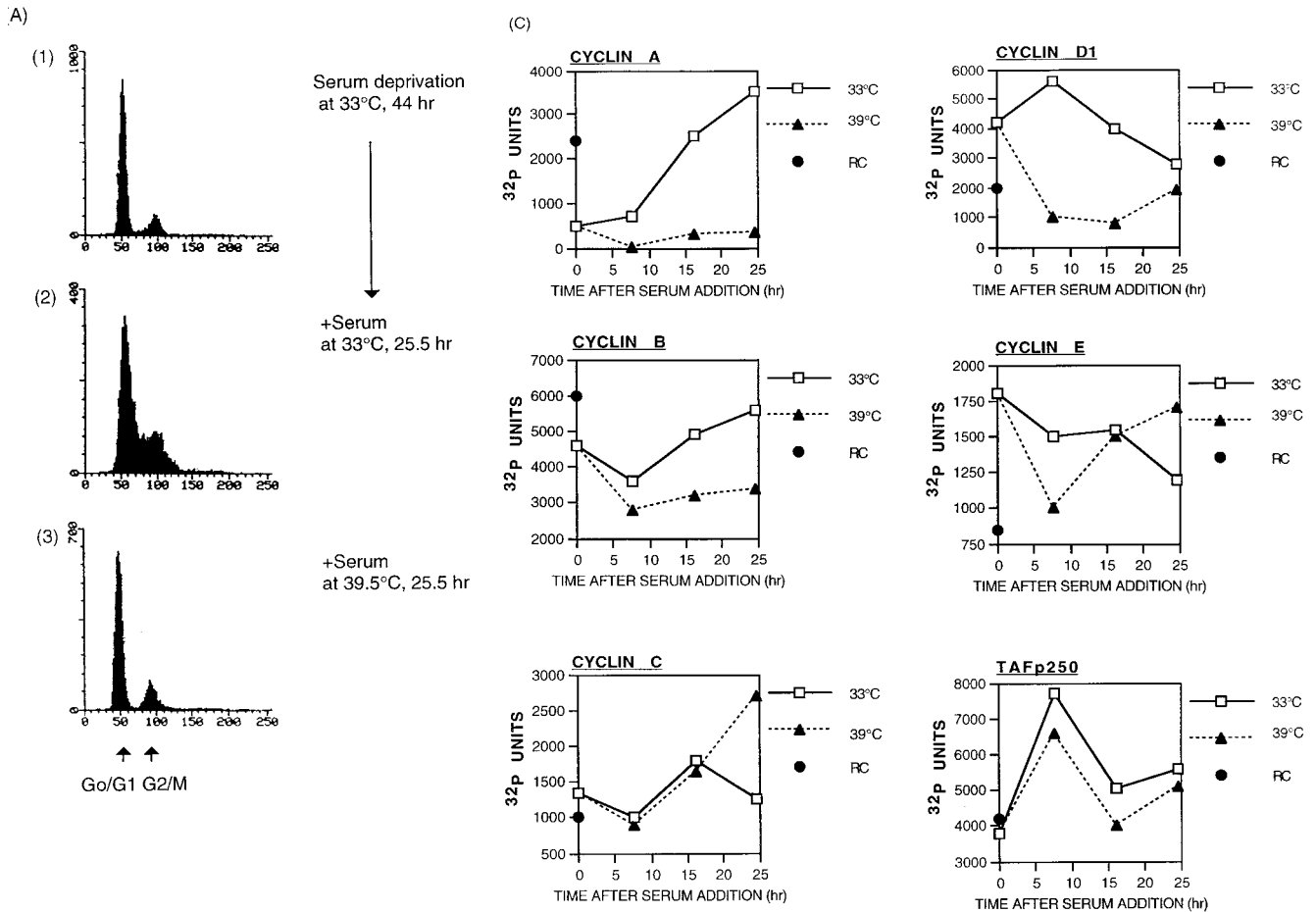


FIG. 1. Transcription rates of specific genes are selectively reduced in synchronized, serum-stimulated ts13 cells at the nonpermissive temperature. (A) Flow cytometric analysis of ts13 cells. The DNA contents of ts13 cell populations were measured by flow cytometry after propidium iodide staining. The G₀/G₁ and G₂/M populations are indicated at the bottom. (1) Cells synchronized by growth in 0.25% serum for 44 h at 33°C. (2) Synchronized cells cultured for 25.5 h at 33°C after 10% serum addition. (3) Synchronized cells cultured for 25.5 h at 39.5°C after 10% serum addition. (B) Nuclear run-on analysis of transcription of various cell cycle-regulated genes in ts13 cells. Shown are autoradiograms of radiolabeled RNAs hybridized to membrane-immobilized cDNA fragments in a slot blot manifold (Schleicher & Schuell). Radiolabeled RNAs were from randomly cultured cells (RC; lane 1), cells synchronized by serum deprivation for 47 h at 33°C (SS; lane 2), synchronized cells grown for an additional 7.5 h after serum addition at 33°C (lane 3) or 39.5°C (lane 4), synchronized cells grown for 16 h after serum addition at 33°C (lane 5) or 39.5°C (lane 6), and synchronized cells grown for 24.5 h after serum addition at 33°C (lane 7) or 39.5°C (lane 8). The cDNA hybridization probes, indicated at the left, are as follows: BS (pBluescript; negative control); CYCA (cyclin A); CYCB (cyclin B); CYCC (cyclin C); CYCD1 (cyclin D1); CYCE (cyclin E); CDK2; E2F1; RB; TAF_{II}250; βACTIN (β-actin); and 5S (5S RNA genes). Arrows at the right indicate the cyclin A and cyclin D1 results. The minor differences observed at 33°C versus 39.5°C for cyclin B and E2F1 were either not significant when the background signal was subtracted or not clearly reproducible in other run-on assays (data not shown). (C) Quantitative analysis of the nuclear run-on assays. The data in panel B were quantitated with a PhosphorImager (Molecular Dynamics). Arbitrary units indicate relative radioactivity. Local backgrounds were subtracted from each of the results. Transcriptional activities are shown for synchronized cells following serum stimulation at 33 or 39°C and for randomly growing cells (RC) at 33°C. The open squares at time zero represent activities in synchronized (serum-deprived) cells at 33°C.

cyclin D2 was considerably lower than that for cyclin D1 or D3. However, at 7.5 h after serum addition, the transcription rate appeared to be modestly increased at 33°C and slightly decreased at 39.5°C, leading to a differential effect (about three-fold) somewhat lower than that observed for the cyclin D1 and D3 genes. We also examined transcription rates in synchronized BHK cells, the wild-type cell from which ts13 was derived, in order to rule out the possibility that the reduced transcription rates at 39.5°C reflected cellular adaptation to the higher temperature from 33°C. Although the induction of cyclin D1 gene transcription was lower than that in ts13 cells, no significant differences in transcription rates were observed for cyclin D1 and D3 genes in BHK cells after 8 h of incubation with serum at 33°C versus 39.5°C (Fig. 2B, lanes 2 and 3). This observation suggests that the reduced transcription of cyclin D1 and D3 genes at 39.5°C in ts13 cells is a result of the mutation in TAF_{II}250.

The results in Fig. 1 and 2 indicate significant and selective reductions in cyclin A and D (D1 and D3) transcription rates at 39.5°C in ts13 cells. To better assess which genes might be directly affected by the TAF_{II}250 mutation, temporal changes in transcription rates were analyzed. In synchronized cells, the cyclin D1 and D3 transcription rates were reduced by 2 h of incubation at 39.5°C following a 6-h incubation at 33°C (data not shown). Further, in normal growing (randomly cultured) cells, the cyclin D1 and D3 transcription rates decreased within 1 h after transfer to 39.5°C, whereas no significant difference in the cyclin A transcription rate was observed after 2 h of incubation at 39.5°C (data not shown). Therefore, the data are consistent with a direct effect of the TAF_{II}250 mutation on cyclin D1 and D3 transcription, as opposed to an effect on transcription of another gene whose product is required for cyclin D1 or D3 transcription, whereas the possibility of a direct effect on cyclin A transcription appears less likely.

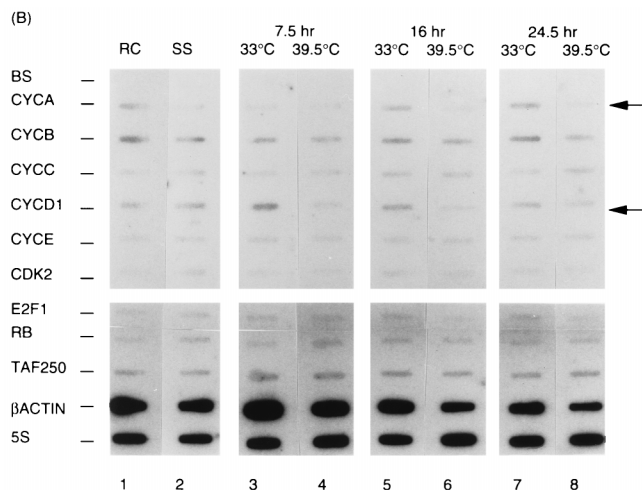


FIG. 1—Continued.

Transcription of a transfected human cyclin D1 gene is selectively reduced in ts13 cells at 39.5°C. There are several important questions regarding the mechanism(s) involved in the reduction of cyclin D1 and D3 transcription in ts13 cells. One is whether the effect of the TAF_{II}250 mutation on cyclin D (D1 and D3) gene transcription is species specific or whether it more generally affects other mammalian cyclin genes. Another is which regions of the promoters are involved in the transcription that is sensitive to the TAF_{II}250 mutation in ts13 cells at 39.5°C. The promoter of the human cyclin D1 gene has been cloned by several groups (21, 44, 49) and is typified by the absence of a TATA box and the presence of a weak initiator element, along with a number of other possible regulatory elements. Although there is some discrepancy between various studies, transcription of the human cyclin D1 gene appears to be stimulated by serum through at least two different factor binding sites for AP-1-related proteins (2, 21). We have used transient-transfection assays with the human cyclin D1 promoter fused to a luciferase reporter gene (21) to investigate these questions. Cells were first transfected with various promoter deletion constructs of the cyclin D1 gene and then synchronized at G₀/G₁ by serum deprivation. After subsequent serum stimulation, cells were grown at 33 or 39.5°C for 16 h and then harvested for assay (Fig. 3).

Relative to the level of activity observed just at the time of serum addition, transcription of the reporter (Δ-973) containing the longest region of the human cyclin D1 promoter was increased about twofold at 33°C but decreased about twofold at 39.5°C, giving an overall difference in activity of three- to fivefold at 33°C versus 39.5°C in ts13 cells (Fig. 3B). A control analysis showed only a minor effect of serum, and no significant effect of temperature, on transcription of an SV40 promoter/enhancer-driven reporter (pSV2A/LΔ5') that is known not to respond to serum stimulation (39), in both ts13 and BHK cells (Fig. 3B and C). In contrast, transcription of the Δ-973 construct of the human cyclin D1 promoter was increased about twofold (again relative to cells analyzed at the time of serum addition) at both 33 and 39.5°C in the parental BHK cells (Fig. 3C). These results are consistent with the results of the above-described analysis of the effects of serum and temperature on transcription rates of the endogenous cyclin D1 gene measured by nuclear run-on assay (Fig. 1 and 2). They confirm a specific effect of the TAF_{II}250 mutation on transcription of the cyclin D1 gene, even when assayed by transfection, and further indi-

cate that the effect of the mutation on cyclin D1 transcription is not species specific. A similar result has recently been reported by Sekiguchi et al. (59).

A more significant increase in transcription in response to serum at 33°C was detected with the Δ-944 construct that still contains both a TPA (12-*O*-tetradecanoylphorbol-13-acetate) response element (TRE) at positions -935 to -929 and a regulatory element for an unknown factor at positions -928 to -921 (Fig. 3A and reference 21). In this case, the ratio of transcription activities in the presence of serum at 33°C versus 39.5°C was 22-fold in ts13 cells (Fig. 3B). Consistent with these results, Herber et al. (21) also observed a greater enhancement of transcription by serum with the Δ-944 template (compared to the Δ-973 template) in NIH 3T3 cells. The greater activity of the Δ-944 construct relative to the Δ-973 construct may reflect the loss of an AT-rich region potentially capable of enhancing formation of a nucleosome-like structure and acting as a transcription silencer in vivo (21). In vitro transcription results support this possibility, since there is no significant difference in activities of the Δ-973 and Δ-944 templates in cell-free systems from HeLa and ts13 cells (Fig. 4A and B).

Constructs containing fewer upstream sequences than the Δ-944 construct showed marginal to insignificant increases in response to serum at 33°C, but in some cases, the activities were still decreased at 39.5°C compared to 33°C. Thus, the activities of the Δ-742 and Δ-254 reporters were reduced about threefold at 39.5°C, while that of Δ-78 was reduced about twofold (Fig. 3B). The Δ-29 reporter, which does not contain any known activator binding sites (Fig. 3A and reference 21), did not show any induction by serum addition or any difference in activity at 39.5°C versus 33°C in ts13 cells (Fig. 3B). In BHK cells, the Δ-29 reporter showed a slight (1.5-fold) induction by serum addition but no difference in activity at 39.5°C versus 33°C (Fig. 3C). These results are consistent with those of the nuclear run-on assays, which suggested that cyclin D1 transcription is directly affected by the TAF_{II}250 mutation. They are also consistent with the results of Herber et al. (21), who analyzed the effects of serum stimulation on the same reporters in NIH 3T3 cells.

TFIID-dependent transcription of the cyclin D1 promoter in vitro. The results from the analyses shown in Fig. 1 to 4 indicate that the TAF_{II}250 mutation in ts13 cells selectively affects cyclin D1 transcription. To analyze the mechanism involved, we established an in vitro transcription system reconstituted with cyclin D1 templates and nuclear extracts prepared from randomly growing HeLa, ts13 and BHK cells.

One notable difference between the HeLa and ts13 or BHK (parental hamster cell line) nuclear extracts was the optimal temperature for transcription. For both cyclin D1 and AdML promoters, transcription in HeLa nuclear extracts was at least five times higher at 30°C than at 20 or 25°C, whereas transcription in ts13 or BHK nuclear extracts was three to five times higher at 20 or 25°C than at 30°C (data not shown). On the basis of these results, the in vitro transcription experiments shown in this study were performed at 20°C for ts13 and BHK nuclear extracts and at 30°C for HeLa nuclear extracts.

In vitro analyses of the cyclin D1 5'-deletion templates used in the transient transfection assays failed to reveal any significant differences in the transcription activities of the Δ-973, Δ-944, and Δ-742 templates in either HeLa (Fig. 4A, lanes 2 to 4) or ts13 (Fig. 4B, lanes 2 to 4) nuclear extracts. These results contrast with the greater activities of the Δ-944 template, relative to the Δ-973 and Δ-742 templates, in transient-transfection assays in synchronized serum-stimulated ts13 (Fig. 3B) and NIH 3T3 (21) cells. This result suggests that there may be a serum-induced activator of cyclin D1 transcription which is

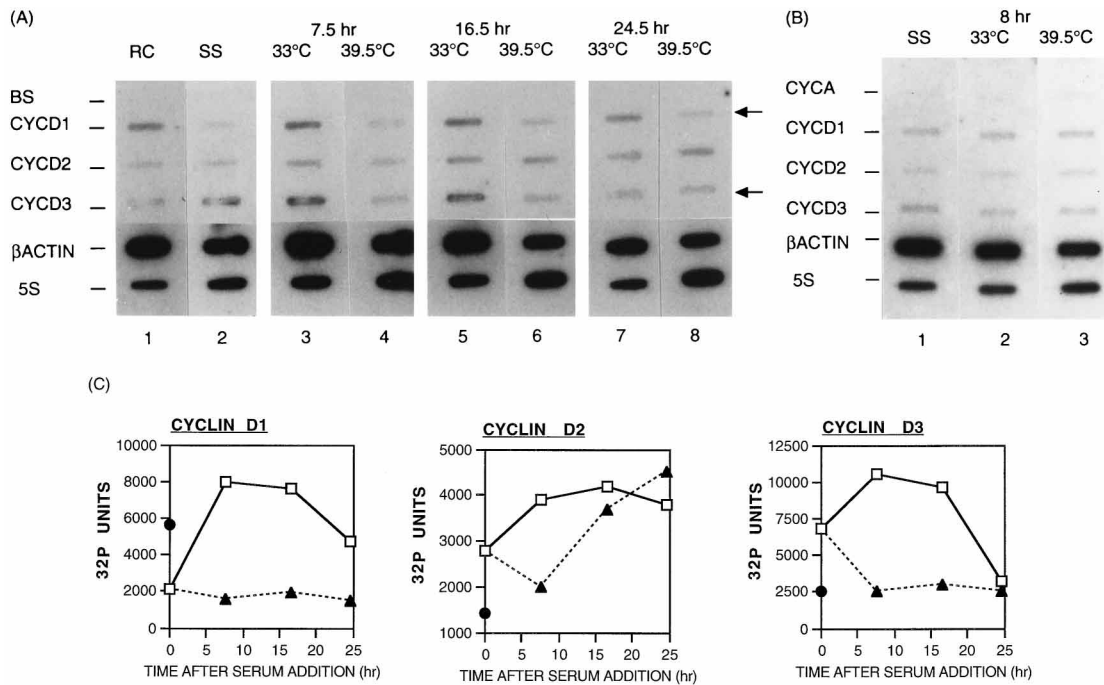


FIG. 2. Transcription rates of cyclin D1 and D3 genes are selectively reduced in synchronized, serum-stimulated ts13 cells, but not in parental BHK cells, at the nonpermissive temperature. (A) Nuclear run-on analysis of D-type cyclin gene transcription in ts13 cells. Experimental conditions, including those for cell synchronization and serum-stimulated growth at permissive and nonpermissive temperatures, were the same as described for Fig. 1B. DNA or cDNA clones corresponding to mouse cyclins D1 (CYCD1), D2 (CYCD2), and D3 (CYCD3) and to β -actin (β ACTIN), 5S RNA genes (5S), and pBluescript (BS) were used as probes in the DNA-RNA hybridization assays as indicated at the left. Radiolabeled RNAs were from randomly cultured cells (RC; lane 1), cells synchronized by serum deprivation for 47 h at 33°C (SS; lane 2), synchronized cells grown for an additional 7.5 h after serum addition at 33°C (lane 3) or 39.5°C (lane 4), synchronized cells grown for 16 h after serum addition at 33°C (lane 5) or 39.5°C (lane 6), and synchronized cells grown for 24.5 h after serum addition at 33°C (lane 7) or 39.5°C (lane 8). (B) Nuclear run-on analysis of cyclin A and D transcription in parental BHK cells. Experimental conditions were as described for Fig. 1B. The autoradiogram shows a hybridization analysis of RNAs from cells synchronized by serum starvation for 47 h at 33°C (SS; lane 1) and from synchronized cells grown for 8 h at 33°C (lane 2) or 39.5°C (lane 3) after serum stimulation. The cDNA hybridization probes indicated at the left include those for cyclin A (CYCA) and other genes indicated in panel A. (C) Quantitative analysis of the nuclear run-on assays. The data in panel A were quantitated with a PhosphorImager (Molecular Dynamics). Arbitrary units indicate relative radioactivity. Transcriptional activities are shown for synchronized cells following serum stimulation at 33°C (open squares) or 39.5°C (solid triangles) and for randomly growing cells at 33°C (solid circles). The open squares at time zero represent activities in synchronized (serum-deprived) cells at 33°C.

active in the transition from resting to growing cells, but not in (extracts from) cycling cells, and dependent on sequences between -944 and -742 . In the *in vitro* assays, transcription activities were decreased two- to threefold following deletion of cyclin D1 promoter sequences between -742 and -254 (Fig. 4, lanes 4 and 5). Further reductions in transcription activity (two- to threefold) were observed with the deletion of sequences between -78 and -29 , while the Δ -29 template was less than 20% as active as the Δ -973 template in both nuclear extracts (Fig. 4, lanes 5 to 7).

Mild heat treatment selectively reduces cyclin D1 promoter transcription in ts13 (but not BHK) nuclear extracts in an activator-specific manner. To further investigate the function of TAF_{II}250 in cyclin D1 transcription, we preincubated nuclear extracts from ts13 and BHK cells at various temperatures in order to selectively inactivate, via the ts13 mutation, specific TFIID functions. We found that transcription of the cyclin D1 promoter (Δ -973 template) was reduced significantly (three- to fivefold) by preincubation of ts13 nuclear extract at either 30 or 40°C for 15 min (mild heat treatment) (Fig. 5B, lanes 6 and 7 versus lane 2), whereas transcription in BHK nuclear extracts was not affected by preincubation at either 30 or 40°C (Fig. 5B, lanes 13 and 14 versus lane 9). Preincubation at 0 or 20°C had no effect on cyclin D1 transcription in either the ts13 extract (Fig. 5B, lanes 4 and 5 versus lane 2) or the BHK extract (Fig. 5B, lanes 11 and 12 versus lane 8). Similar results were observed with the Δ -944 template (data not shown). Preincuba-

tion of the ts13 nuclear extract at 30°C for 15 min did not affect either basal transcription (Fig. 5C, lane 3 versus lane 2; see also the figure legend) or GAL4-VP16-activated transcription (Fig. 5C, lanes 7 and 8 versus lanes 5 and 6) on the AdML promoter.

To further prove that heat-treated ts13 extracts are defective in TFIID function, we tested their ability to complement a HeLa nuclear extract heated at 47°C for 12.5 min. This treatment was shown previously to selectively inactivate TFIID (45) and markedly inhibited the ability of HeLa nuclear extracts to transcribe the cyclin D1 promoter (Fig. 6A, lane 3 versus lane 2). Cyclin D1 transcription in this extract was restored both by addition of an affinity-purified FLAG epitope-tagged human TFIID (fTFIID [Fig. 6A, lanes 4 to 6]) and by ts13 nuclear extract (Fig. 6A, lane 8) but not by mildly heat-treated (33°C, 15 min) ts13 nuclear extract (Fig. 6A, lane 7). The low amounts of ts13 nuclear extracts added to the inactivated HeLa extract were insufficient, by themselves, to effect significant cyclin D1 transcription (Fig. 6A, lanes 9 and 10). In contrast to TFIID, recombinant human TBP was unable to restore cyclin D1 transcription to the HeLa extract (data not shown). Addition of fTFIID to mildly heat-treated ts13 extracts also restored their ability to transcribe the cyclin D1 promoter (Fig. 6B, lanes 1 to 4) but had little effect on cyclin D1 transcription in control ts13 extracts (Fig. 6B, lanes 5 to 8).

These results suggest that the transcription reduction in mildly heat-treated ts13 nuclear extract is directly related to a defect in the function of endogenous TFIID and further em-

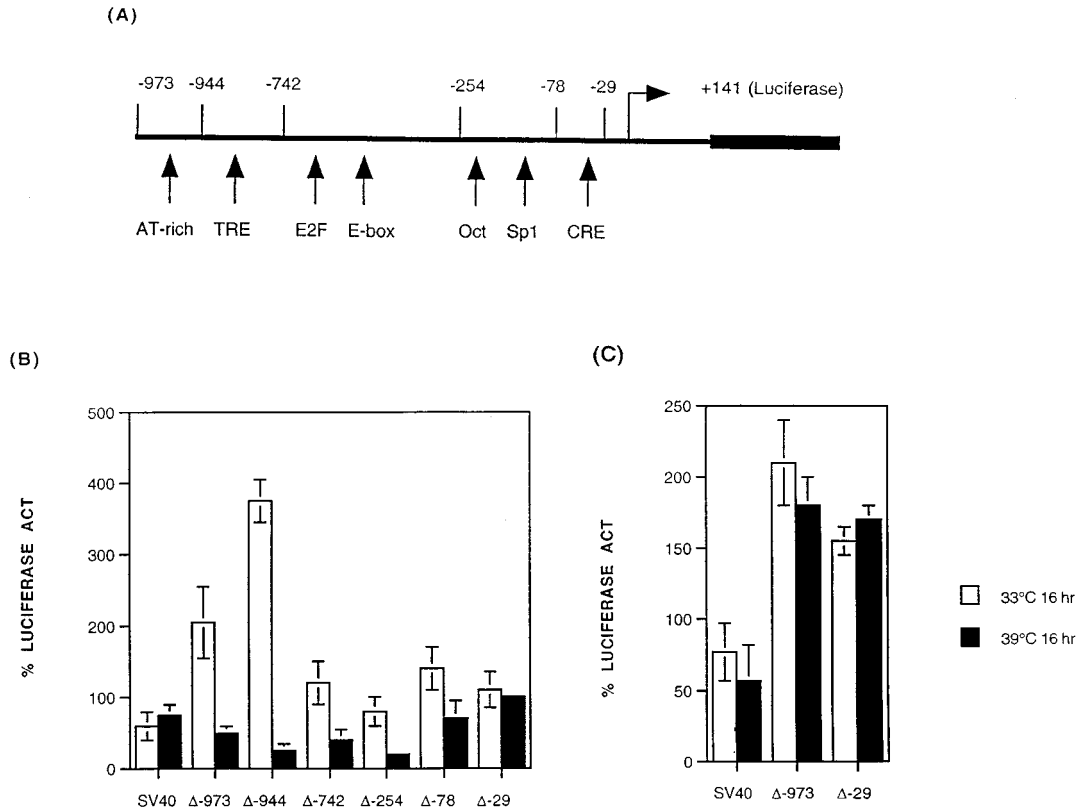


FIG. 3. Serum-induced transcription of a transfected human cyclin D1 gene is reduced at the nonpermissive temperature in ts13 cells but not in BHK cells. (A) Schematic of the intact and 5'-deleted human cyclin D1 promoter-luciferase constructs (21). Numbers at the top indicate the endpoints of the 5'-deletion mutants. The transcription initiation site is shown as +1. DNA elements and consensus binding sites for transcription factors are shown with arrows. The arrows indicate consensus binding sequences for transcription factors. E-box, a site for helix-loop-helix proteins. (B) Transient-transfection assays of luciferase reporter genes driven by 5'-deletion mutants of the human cyclin D1 promoter in ts13 cells. ts13 cells were transfected with the control SV40 promoter/enhancer construct (pSV2A/LΔ5') and the human cyclin D1 promoter constructs Δ-973, Δ-944, Δ-742, Δ-254, Δ-78, and Δ-29 (see panel A). Luciferase activities were measured in synchronized cells following serum stimulation for 16 h at 33°C or 39°C and are normalized to the luciferase activities (set at 100%) determined in synchronized cells maintained at 33°C under serum deprivation conditions. (C) Transient-transfection assays of luciferase reporter genes driven by human cyclin D1 promoters in BHK cells. BHK cells were transfected with the control SV40 promoter/enhancer construct (pSV2A/LΔ5') and the human cyclin D1 promoter constructs Δ-973 and Δ-29. Luciferase activities were measured in synchronized cells maintained at 33°C for 16 h under serum deprivation conditions (100% control values) and in synchronized cells following growth in serum for 16 h at 33°C or at 39°C.

phasize a TAF requirement for cyclin D1 transcription, as shown previously for other natural mammalian promoters containing initiator elements but lacking TATA boxes (40, 64). The results shown in Fig. 5 and 6 strongly suggest that the TAF_{II}250 mutation in ts13 cells affects cyclin D1 transcription in a direct and promoter-specific manner.

We next wished to determine whether the TAF_{II}250 mutation affects TFIID function in basal transcription on the cyclin D1 promoter, thereby having an indirect effect on the function of upstream activators, or whether the mutation has specific, and more direct, effects on functional interactions of upstream activators with core promoter factors. For this analysis, we used a template with five GAL4 sites upstream of the cyclin D1 core promoter region (-29 to +139) (Fig. 7B). This approach was taken because the natural upstream activators on the cyclin D1 promoter are not well defined and because the basal activity of the cyclin D1 core promoter is too low even in control ts13 extracts (Fig. 7A, lanes 3 and 8) to reliably assess effects of preincubation at the nonpermissive temperature. On the modified cyclin D1 templates, the GAL4 DNA binding domain-activation domain fusion proteins GAL4-p65, GAL4-p53, and GAL4-VP16 activated transcription to significant but variable levels, but for a given activator, the level of activation

was the same for control and preincubated ts13 nuclear extracts (Fig. 7A, lanes 4 and 5 versus lane 3, lanes 6 and 7 versus lane 3, and lanes 9 and 10 versus lane 8). Activation by GAL4-Sp1 also was unaffected by preincubation of the ts13 nuclear extract (data not shown). If the intrinsic capacity of the general factors to mediate basal transcription from the cyclin D1 promoter had been reduced by mild heat treatment (33°C for 15 min), then activation of transcription by any of the activators might have been reduced by the preincubation. Along with the results of Fig. 4B, these data thus suggest that the ts13 mutation does not necessarily affect the basal functions of the cyclin D1 promoter and its interacting factors (including TFIID) but does affect the function of an upstream activator(s) that is specific for the natural cyclin D1 promoter and that presumably interacts with TFIID (or the TFIID-containing preinitiation complex) in a unique manner.

DISCUSSION

Although in vitro biochemical studies of human and *Drosophila* TFIID have provided evidence for the role of individual TAFs as direct or indirect targets for specific activators (see the introduction), there has been little genetic evidence to

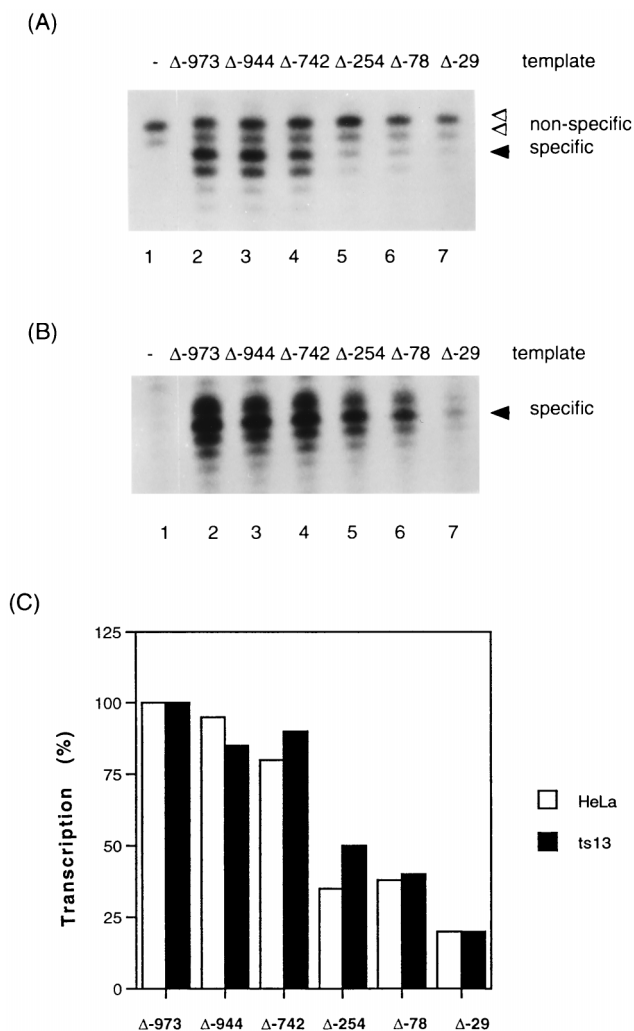


FIG. 4. Transcription of the human cyclin D1 promoter in HeLa and ts13 nuclear extracts. (A) Effects of 5' deletions on transcription of the human cyclin D1 promoter in a HeLa nuclear extract. Transcription reaction mixtures were incubated at 30°C for 1 h either without template (lane 1) or with 100 ng of cyclin D1 promoter constructs Δ -973 (lane 2), Δ -944 (lane 3), Δ -742 (lane 4), Δ -254 (lane 5), Δ -78 (lane 6), and Δ -29 (lane 7). Accurately initiated transcripts from the major +1 initiation site on the cyclin D1 template were monitored by primer extension. The major specific transcript is marked with a solid arrowhead. Non-specific bands that are visible even in the absence of template (lane 1) are marked with open arrowheads. (B) Effects of 5' deletions on transcription of the human cyclin D1 promoter in ts13 nuclear extract. Transcription reaction mixtures were incubated at 20°C for 1 h either without template (lane 1) or with 100 ng of cyclin D1 promoter constructs Δ -973 (lane 2), Δ -944 (lane 3), Δ -742 (lane 4), Δ -254 (lane 5), Δ -78 (lane 6), and Δ -29 (lane 7). The position of the major specific (+1) cyclin D1 transcript is indicated by a closed arrowhead. The bands surrounding the +1 transcript may represent either heterogeneous initiation events around the +1 site or incompletely extended products of the primer extension assay. (C) Quantitative analysis of effects of 5' deletions on human cyclin D1 promoter transcription in HeLa and ts13 nuclear extracts. The data from panels A and B were quantitated with a PhosphorImager (Molecular Dynamics). The relative transcription activities (normalized to the Δ -973 activity) of the 5' deletion mutants are shown.

confirm this. On the other hand, while a gene-specific regulatory role for TAF_{II}250 (or a domain thereof) was indicated by a combination of the earlier genetic studies of CCG1 and the later biochemical analyses of TAF_{II}250 (see the introduction), the lack of information regarding specific genes and corresponding regulatory factors directly affected by the ts13 mutation in CCG1/TAF_{II}250 has precluded biochemical studies of

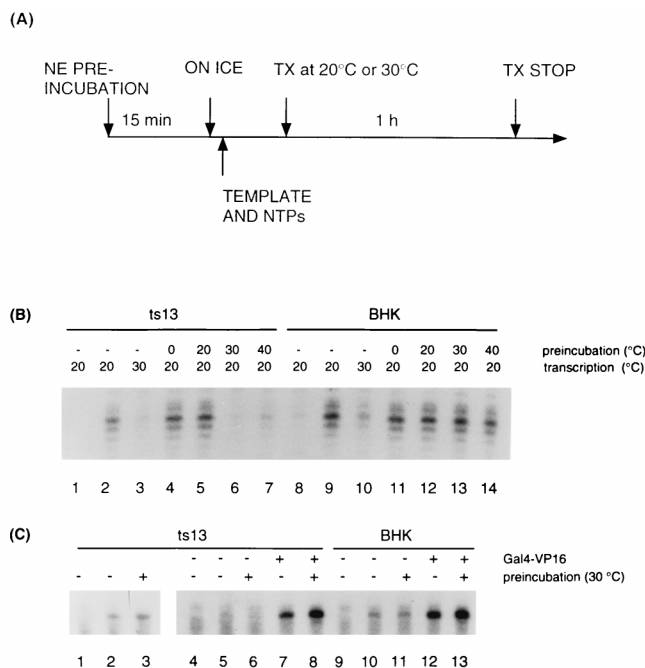


FIG. 5. Transcription of the cyclin D1 promoter is selectively reduced by mild heat treatment of ts13 extracts but is unaffected by mild heat treatment of control BHK extracts. (A) Schematic of the procedure for nuclear extract (NE) preincubation and in vitro transcription (TX) assays. (B) Transcription of the cyclin D1 promoter in ts13 and BHK nuclear extracts. Transcription assays conducted according to the protocol in panel A used ts13 extracts (lanes 1 to 7) or BHK extracts (lanes 8 to 14) and contained either no template (lanes 1 and 8) or 100 ng of the plasmid D1 Δ -973pXP2 (lanes 2 to 7 and 9 to 14). In control assays with no preincubation of nuclear extracts, transcription was carried out at 20°C (lanes 1, 2, and 9) or 30°C (lanes 3 and 10). In assays with preincubated extracts, transcription reactions were conducted at 20°C with nuclear extracts that had been preincubated at 0°C (lanes 4 and 11), 20°C (lanes 5 and 12), 30°C (lanes 6 and 13), and 40°C (lanes 7 and 14). (C) Basal and activator-mediated transcription from the AdML promoter in ts13 and BHK nuclear extracts. Transcription reaction were conducted with ts13 (lanes 1 to 8) and BHK (lanes 9 to 13) extracts, either with (lanes 3, 6, 8, 11, and 13) or without (lanes 1, 2, 4, 5, 7, 9, 10, and 12) preincubation at 30°C and either with (lanes 7, 8, 12, and 13) or without (lanes 1 to 6 and 9 to 11) addition of 200 ng of GAL4-VP16. Transcription reaction mixtures contained either no template (lanes 1, 4, and 9) or 100 ng (lanes 2 and 3) or 50 ng (lanes 5 to 8 and 10 to 13) of a template (5GALAML) with five GAL4 binding sites upstream of the AdML core promoter and were incubated at 20°C for 1 h. Transcripts were analyzed by primer extension.

the mechanisms (factor interactions) involved. In the present study, using the ts13 cell line in conjunction with both in vivo and in vitro transcription analyses, we show that transcription of the cyclin D1 gene is regulated directly through TAF_{II}250. These and other studies of cyclin D1 promoter elements and regulatory factors (2, 21) represent an important step in investigating gene-specific regulatory functions of TAF_{II}250 and, since cyclin D1 is a proto-oncoprotein with an important function at the restriction point for the G₁/S transition (61), have important implications for cell cycle control.

Cyclin D1 gene transcription is directly regulated through TAF_{II}250. As an initial step in the search for genes directly regulated through TAF_{II}250/CCG1, we measured the in vivo transcription rates (in ts13 cells) of several endogenous genes which are required for cell cycle regulation in synchronized ts13 cells. The results indicate that the transcription rates of endogenous cyclin D1 and D3 genes were reduced (three- to fivefold) at 39.5°C relative to 33°C in early/mid-G₁ phase (within 8 h of serum stimulation). Transcription rates of other candidate genes tested (e.g., genes encoding cyclins A, C, and E)

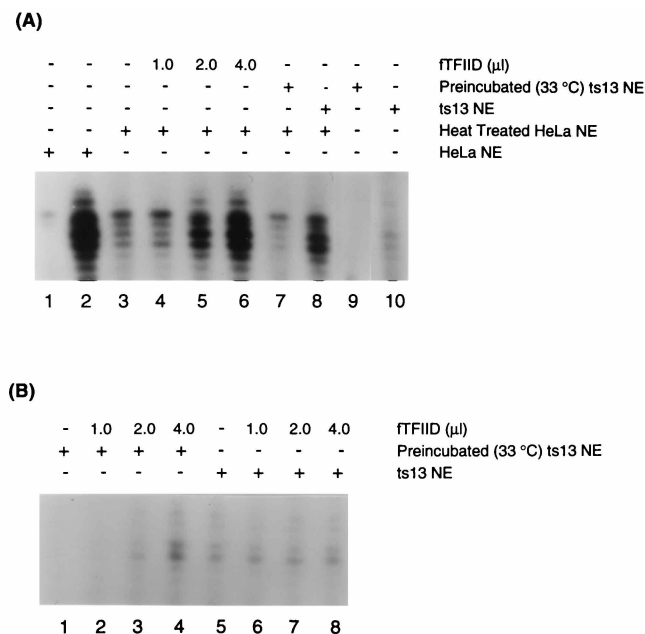


FIG. 6. Addition of TFIID can restore cyclin D1 transcription in mildly heat-treated ts13 nuclear extracts. (A) Transcription of the human cyclin D1 promoter requires TFIID. Assays were conducted at 20°C for 1 h with control and heat-treated HeLa nuclear extracts (NE) supplemented with either affinity-purified TFIID or mildly heat-treated (33°C, 15 min) or nontreated ts13 nuclear extracts. Transcription reactions contained either no template (lane 1) or the Δ -973 template (lanes 2 to 10). Lanes 1 and 2, control HeLa extract; lane 3, heat-treated extract; lanes 4 to 6, heat-treated HeLa extract plus TFIID which contains 4, 8, and 16 ng of FTBP, respectively; lane 7, heat-treated HeLa extract plus mildly heat-treated ts13 extract; lane 8, heat-treated HeLa extract plus control ts13 extract; lane 9, mildly heat-treated ts13 extract; lane 10, control ts13 extract. (B) Transcriptional reduction of the cyclin D1 promoter in mildly heat-treated ts13 nuclear extract can be recovered by addition of human TFIID. Assays were conducted at 20°C for 1 h with mildly heat-treated and control ts13 nuclear extracts supplemented with affinity-purified TFIID. All reaction mixtures contained the Δ -973 template. Lanes 1 to 4, mildly heat-treated ts13 plus TFIID which contains 4, 8, and 16 ng of FTBP, respectively; lanes 5 to 8, control extract plus TFIID which contains 4, 8, and 16 ng of FTBP, respectively.

were not changed within 8 h. In addition, cyclin D1 and D3 transcription levels were reduced rapidly by the temperature shift in both synchronized cells (within 2 h at 39.5°C following 6 h of stimulation with serum at 33°C) and normally growing cells (after 1 h of incubation at 39.5°C) (data not shown). In further support of this idea, the present study and a recent report by Sekiguchi et al. (59) demonstrate that the transcription of an exogenous human cyclin D1 gene is reduced at 39.5°C relative to 33°C in synchronized ts13 (or tsBN462) cells but not in BHK cells.

To gain further evidence for a direct effect of the TAF_{II}250 mutation on the identified target gene(s), we analyzed the temperature sensitivity of cyclin D1 promoter transcription in nuclear extracts from ts13 and BHK cells. Significantly, transcription of the cyclin D1 promoter was reduced three- to fivefold by preincubation of the ts13 nuclear extract at 30 to 40°C for 15 min. This mild heat treatment had no effect on basal or activator (GAL4-VP16)-mediated transcription from the AdML promoter in ts13 extracts, nor did it affect transcription of the cyclin D1 promoter in control nuclear extracts from BHK cells (which contain wild-type TAF_{II}250). Moreover, this transcription reduction was restored by addition of an essentially homogeneous FLAG epitope-tagged and affinity-purified human TFIID. Similarly, mild heat treatment reduced the ability of ts13 extracts, but not BHK extracts, to mediate cyclin D1

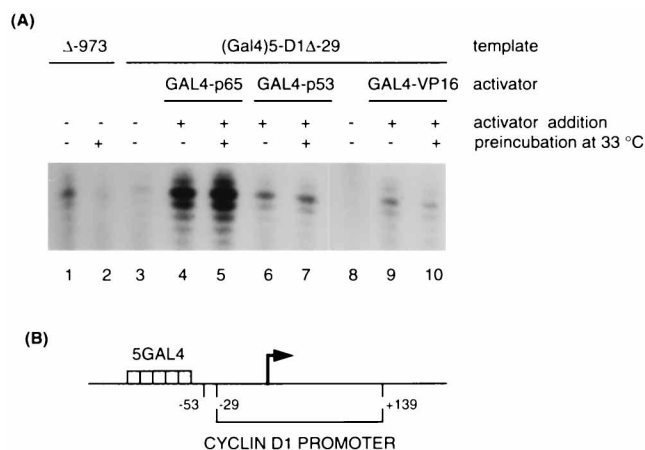


FIG. 7. The ts13 mutation in TAF_{II}250 affects specific activator functions on the cyclin D1 promoter. (A) Various GAL4 activation domain fusion proteins activate transcription of the cyclin D1 promoter in ts13 nuclear extracts at the nonpermissive temperature. Transcription reaction mixtures contained either control ts13 nuclear extracts (lanes 1, 3, 4, 6, 8, and 9) or preincubated (15 min at 33°C) ts13 extracts (lanes 2, 5, 7, and 10), 100 ng of either the Δ -973 cyclin D1 promoter template (lanes 1 and 2) or a hybrid template (5GAL4D1 Δ -29) with five GAL4 sites upstream of the cyclin D1 core promoter (lanes 3 to 10), and either no activator (lanes 1 to 3 and 8) or 15 to 25 ng of GAL4-p65 (lanes 4 and 5), GAL4-p53 (lanes 6 and 7), or GAL4-VP16 (lanes 9 and 10). Reaction mixtures were incubated for 1 h at 20°C prior to transcript analysis by primer extension. (B) Schematic of the template (5GAL4D1 Δ -29).

transcription in a TFIID-dependent complementation assay. Therefore, the promoter-specific transcription reduction of cyclin D1 genes observed in the *in vivo* experiments is reproducible in an *in vitro* system in which indirect effects of the ts13 mutation (e.g., on the expression of genes encoding factors involved in cyclin D1 gene transcription) are unlikely. Moreover, although the current *in vitro* studies have given clear results, they were performed with mildly heat-treated nuclear extracts from normally growing (unsynchronized) ts13 cells. Thus, although presently not technically feasible, experiments with nuclear extracts prepared from synchronized cells could show even more dramatic differences. Altogether, the *in vivo* and *in vitro* results presented here strongly indicate that transcription of the cyclin D1 promoter is directly regulated through TAF_{II}250. Although transcription from the less active cyclin D3 promoter has not yet been analyzed *in vitro*, the promoter structures and cognate activators of the human cyclin D3 and D1 genes appear similar (5). This observation suggests that the two promoters may be regulated directly through TAF_{II}250 by similar mechanisms.

Although a temperature-dependent difference in transcription of the endogenous cyclin A was observed in synchronized serum-stimulated ts13 cells, this was a late response. In contrast to the results observed for cyclin D1 transcription, there was no rapid reduction (within 2 h) of cyclin A transcription in normally growing ts13 cells after a shift to 39.5°C (data not shown). Therefore, it is not yet clear that the observed effect on cyclin A transcription reflects direct effects of the TAF_{II}250 mutation. In this regard, the results of transient-transfection assays by Wang and Tjian (69) also did not demonstrate that the TAF_{II}250 mutation directly affected cyclin A transcription.

Possible mechanisms for transcription regulation of cyclin D1 gene through TAF_{II}250. The TAF_{II}250 mutation in ts13 cells is a single nucleotide transition resulting in a glycine-to-asparagine change at residue 690 (19). This mutation affects cyclin D1 transcription in a promoter-specific manner both in

vivo and in vitro. Thus, in contrast to the results obtained with the intact cyclin D1 promoter, basal transcription from the AdML promoter and transcription from a modified cyclin D1 promoter (with GAL4 sites attached to the cyclin D1 core promoter) in response to various GAL4-based activators were not affected by mild heat treatment of ts13 nuclear extracts. These results indicate that the TAF_{II}250 mutation does not significantly reduce the function of the general initiation factors (including TFIID) on either the AdML or the cyclin D1 core promoter, at least in response to certain activators. Therefore, the TAF_{II}250 mutation most likely affects the function of a specific activator(s) or associated coactivator(s) that interacts directly with TFIID (either with TAF_{II}250 itself or possibly with another TAF whose conformation and/or function within TFIID is in turn affected by the TAF_{II}250 mutation).

Past and present studies of cyclin D1 transcription have indicated an involvement of TRE sequences at positions -935 to -920 and cyclic AMP response element (CRE) sequences at positions -52 to -45 (2, 21). However, the following observations suggest that the factor involved in serum induction through the region between -944 to -742 in the cyclin D1 promoter is not necessarily the specific (or exclusive) activator directly affected by the TAF_{II}250 mutation. First, transient transfection assays showed that the activity of an ectopic human cyclin D1 gene is reduced at 39.5°C with and without the serum-inducible element. This finding suggests that at least one DNA element for an activator directly affected by the TAF_{II}250 mutation is located downstream from the TRE involved in serum stimulation (2, 21). It also suggests that serum stimulation may enhance transcription in a manner additive or synergistic with transcription activation through TAF_{II}250. Second, in vitro transcription levels from the cyclin D1 promoter were almost the same for Δ -944 and Δ -742 templates (with and without the upstream serum-inducible element, respectively) with ts13 nuclear extracts prepared from normally growing cells. This result indicates either that nuclear extracts do not contain a sufficient amount of the factor responsible for the in vivo serum stimulation or that another activity saturates promoter function under the in vitro assay conditions. Nevertheless, the fact that transcription from the template (Δ -742) lacking the in vivo responsive element still is significantly reduced by mild heat treatment of the ts13 nuclear extracts (data not shown) indicates the involvement of a distinct cyclin D1 promoter element, and associated factor(s), whose function is compromised by the TAF_{II}250 mutation.

The upstream activators required for the regulation of cyclin D1 transcription are not yet characterized; therefore, a detailed analysis of their role in TAF_{II}250-dependent cyclin D1 transcription is not yet possible. However, one group of activators potentially involved in TAF_{II}250-mediated transcriptional activation of the cyclin D1 promoter is the AP-1 superfamily of proteins. It has been reported that AP-1 proteins bind the CRE site (around -50) and that cotransfection of c-Jun can enhance transcription through this site (21). Consistent with this result, preliminary in vitro transcription-oligonucleotide competition assays also have suggested that the CRE is essential for optimal cyclin D1 transcription (64a). Similarly, the results of transient-transfection assays in the present study indicate that the cyclin D1 promoter completely loses the temperature-sensitive transcription upon deletion of the CRE. Interestingly, the cyclin D3 promoter also has a CRE around -50 (5), suggesting that the specific location of this element could also have a role in the TAF_{II}250-dependent regulation. Given that various members of the AP-1 family can bind CREs (18), both as homodimers and in various heterodimeric combinations, the actual factor active on the cyclin D1 and D3

CREs is currently uncertain. The actual mechanisms of activation of these factors also are uncertain. However, like other activators shown to engage in specific TAF interactions (reviewed in reference 6), certain of these factors might also engage in direct TAF_{II}250 (or other) interactions that are sensitive to the TAF_{II}250 mutation. Specific cofactors such as CBP (11), known to interact both with CREB (11, 31) and with c-Jun (3), might also be required for activation through TAF_{II}250.

The cyclin D1 core promoter. In addition to specific activator(s), differences in core promoter structures and corresponding preinitiation complexes (reviewed in reference 53) might also account for differential responses to the TAF_{II}250 mutation. Thus, although the promoter of the immediate-early *c-fos* gene has related upstream activator binding sites (ATF and CRE sites around -60; AP-1 and serum response element sites around -300) (14), transcription of the *c-fos* gene is reported to be unaffected at 39.5°C in ts13 cells (29, 46, 69). One potentially relevant difference between *c-fos* and cyclin D1 promoters is that the human cyclin D1 promoter contains a possible initiator (63) but lacks a consensus TATA-box sequence (TATAAA), whereas the *c-fos* promoter has a potential TATA box (14). This observation raises the possibility that the specific core promoter structure, especially the presence or absence of a TATA box, may determine the dependency on certain interactions affected by the TAF_{II}250 mutation. Our demonstration that transcription of the cyclin D1 core promoter requires TFIID (i.e., both TAFs and TBP) is consistent with results described for other TATA-less promoters (40, 64). Interactions of basal factors on TATA-less promoters, including the cyclin D1 promoter, may be weaker and qualitatively different from those on TATA-containing promoters, since TBP cannot recruit basal factors through interaction with a TATA box (40). Therefore, altered interactions between TAF_{II}250 and a specific activator may have promoter-specific consequences for transcription activation.

Cyclin D1 and cell cycle progression in ts13 cells. Serum-starved ts13 cells are arrested in late G₁ phase (3 to 3.5 h before entry into S phase) following synchronization either in G₀/G₁ by serum deprivation or in early G₁ by isoleucine deprivation or mitotic cell detachment (15, 65). This finding indicates that a major defect in ts13 cells is the lack of some function(s) required in the early/mid-G₁ phase of the cell cycle. Cyclin D is an early/mid-G₁-induced gene and is of key importance for the G₁/S transition (41). It is possible that reductions in the levels of D cyclins directly reduce the levels of RB phosphorylation in vivo. In fact, the RB-stimulated Sp1-mediated transcription activation (GAL4-Sp1) of the E1B (TATA-box-containing) promoter through five GAL4 sites upstream was reported to be reduced about twofold in ts13 cells at 39.5°C (60). It also is possible that transcription of other genes required for the G₁/S transition (e.g., genes encoding cyclin inhibitors p16, p21, and p27 [reviewed in reference 62]), which were not examined in this study, are directly or indirectly affected by the TAF_{II}250 mutation in ts13 cells (59). Taken together, alteration of these cell cycle-regulatory functions might force ts13 cells to arrest in late G₁ phase.

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