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23. The GAL4 DBD included sequences (MKLLSSIEQACDICRLLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLLTEVESRLERLEQLFLLIFPREDLDMILKMDSLQDIKALL). The VP16 activation domain included sequences (APITDVSLGDELRLDGEEVDMTPADALDDFDLEMLGDVESPSPGMTHDPVSYGALDVDDFEFEQMFTDAMGIDDFGG). The GR moiety included sequences (MGLYMGETETKVMGNDLGYPQQAMGIDDFGG). The sequences of the boundaries were the following: [GAL4]-VP16; GTPAAASTQFPGIW; [GAL4]-GR, GTPAAASTLARGS; GR-VP16, GRAPQFPGIW; linkers flanking homopolymers were LEED (amino neighbors) and GDRYP (carboxy neighbors), respectively.
24. We thank D. Arnosti for HeLa nuclear extracts and C. Hovens and J. Silke for critical reading of the manuscript. We thank M. Gstaiger for helpful discussions. The preparation of the figures by F. Ochsenbein is gratefully acknowledged.

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Promoter-Selective Transcriptional Defect in Cell Cycle Mutant ts13 Rescued by hTAF$_{II}$250

Edith H. Wang and Robert Tjian

The TAF$_{II}$250 subunit of the human transcription factor IID (TFIID) rescues the temperature-sensitive hamster cell line ts13 and overcomes a G$_1$ arrest. Investigation of the transcriptional properties of ts13 nuclear extracts in vitro showed that activation by the site-specific regulators Sp1 and Gal4VP16 is temperature sensitive in ts13 extracts, whereas basal transcription remains unaffected. This transcriptional defect can be rescued by purified human TFIID or by expression of wild-type TAF$_{II}$250 in ts13 cells. Expression from the cyclin A but not c-fos promoter is temperature sensitive in these mutant cells. Thus, the mutation in TAF$_{II}$250 appears to have gene-specific effects that may lead to the ts13 cell cycle phenotype.

The eukaryotic cell cycle is a highly regulated process that is dependent on the temporal expression of specific genes (*1*). The cloning of TAF$_{II}$250 (*2*, *3*), a subunit of the RNA polymerase II transcription factor TFIID (*4*, *5*), has provided a link between cell cycle regulation and the general transcriptional machinery. This largest subunit of human TFIID is encoded by CCG1 (*6*, *7*), a gene that overcomes a G$_1$ arrest in the temperature-sensitive hamster cell line, ts13 (*8*). The finding that TAF$_{II}$250 may play a role in cell cycle regulation prompted us to examine the transcriptional properties of ts13 cells. In particular, we focused on transcriptional activation because TAFs have been postulated to represent a class of factors termed coactivators that are required for regulated but not basal transcription (*4*, *5*, *9*, *10*). The ts13 cells do not exhibit a global defect in mRNA synthesis at the nonpermissive temperature (*11*). Thus, the temperature-sensitive cell cycle block in these cells may result from the altered expression of a discrete set of genes.

We have used the mutant hamster cell line ts13 to investigate the potential temperature-sensitive and promoter-specific behavior of the mammalian transcription factor TAF$_{II}$250 in vitro and in vivo. In addition, we hoped to obtain evidence that TAFs indeed act to mediate transcriptional activation in the context of intact cells. In order to study regulated transcription in these mutant cells, we used two well-characterized transcriptional activators, Gal4VP16 and Sp1. Because the mechanism of temperature sensitivity in ts13 cells is unknown, we prepared nuclear extracts from cells grown under permissive (33.5°C) as well as nonpermissive (39.5°C) conditions (*12*) and examined their transcriptional properties in vitro (Fig. 1A). All extracts supported basal transcription. In contrast, nuclear extracts derived from ts13 cells grown at either temperature were defective for Gal4VP16- and Sp1-mediated activation when assayed under conventional assay conditions. As a control, we found that activation was normal in extracts prepared from the parental wild-type BHK-21 hamster cells (Fig. 1A). These results were somewhat unexpected because ts13 cells maintained at 33.5°C presumably contain the functional form of TAF$_{II}$250. We confirmed by protein immunoblot analysis that TAF$_{II}$250 is expressed and appears to be intact in ts13 cells grown at both the permissive and nonpermissive temperatures (Fig. 1B). However, it is possible that the transcriptional machinery in ts13 cells is defective for activation when assayed in vitro under standard conditions of 30°C. We therefore performed in vitro transcription at lower temperatures. At 20°C, transcriptional activation was restored in nuclear extracts from ts13 cells (Fig. 2A, lanes 7, 8, 13, 14, and 2B). The levels of activation decreased two- to threefold as the reaction temperature was increased from 20° to 25°C, and became virtually undetectable at 30°C (Fig. 2, A and B), whereas basal transcription remained unchanged or actually increased somewhat at the higher temperature. In contrast, nuclear extracts from BHK-21 cells exhibited comparable levels of activation at all temperatures (Fig. 2A, lanes 1 to 6, and 2B). Therefore, in ts13 extracts, activated but not basal transcription is temperature sensitive in vitro.

To obtain more direct evidence that TAF$_{II}$250 and therefore TFIID is functionally impaired in ts13 cells, we asked whether human TFIID could restore transcriptional activation in ts13 extracts. Wild-type TFIID from HeLa cells was affinity-purified from the 1.0 M KCl phosphocellulose fraction of LTRα3 cells as described (*10*) and added to extracts from

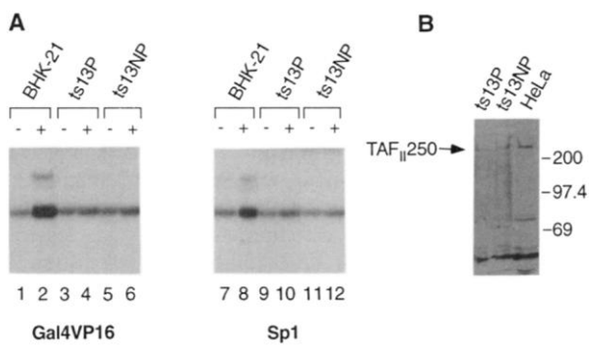

Fig. 1. The ts13 nuclear extracts are defective at 30°C for transcriptional activation in vitro. (**A**) Transcription reactions (*25*) performed in nuclear extracts prepared from BHK-21 cells (lanes 1, 2, 7, and 8), ts13 cells grown at 33.5°C (lanes 3, 4, 9, and 10), and ts13 cells shifted to 39.5°C (lanes 5, 6, 11, 12) were incubated at 30°C in the absence (−) or presence (+) of Gal4VP16 (lanes 1 to 6) or Sp1 (lanes 7 to 12). The DNA templates used contain the adenovirus 5 E4 promoter downstream of either five Gal4-binding sites (lanes 1 to 6) or six GC boxes from SV40 (lanes 7 to 12). Reaction products were detected by primer extension, separated on 8% denaturing polyacrylamide gel, and subjected to autoradiography. (**B**) Full-length TAF$_{II}$250 is expressed in ts13 cells maintained at the permissive and nonpermissive temperatures. Nuclear extracts (200 μg) from ts13 cells grown at 33.5°C (ts13P) at 39.5°C (ts13NP), and from HeLa cells were separated on 6% SDS-polyacrylamide gel and subjected to enhanced chemiluminescence protein immunoblot analysis. The antibody used was a polyclonal antiserum to residues 568 to 590 of human TAF$_{II}$250, a peptide that is 100% conversed between the human and hamster proteins (*26*). The positions of TAF$_{II}$250 and molecular size standards (Bio-Rad) are indicated on the left and right (in kilodaltons), respectively.

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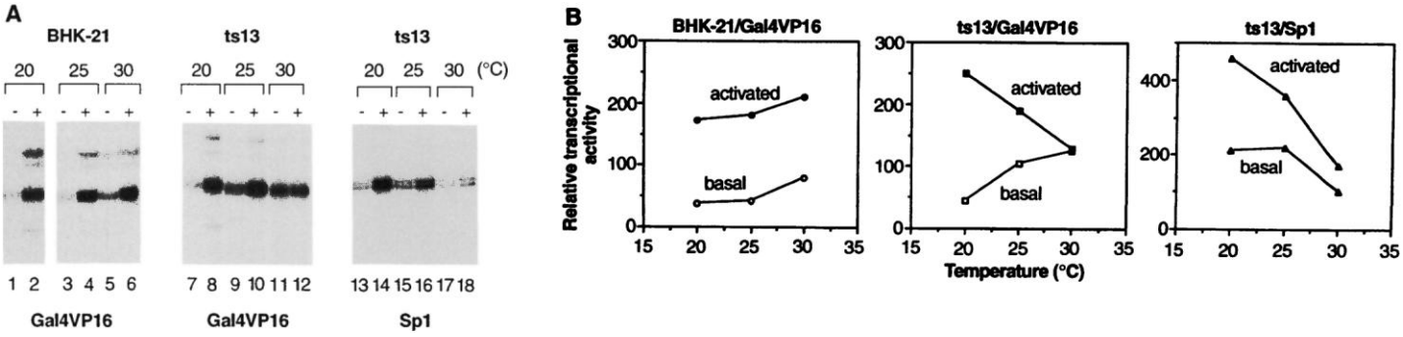


Fig. 2. Temperature-sensitive transcriptional activation in ts13 nuclear extract. (**A**) Activated transcription in ts13 nuclear extracts is temperature sensitive in vitro. Transcription reactions in BHK-21 (lanes 1 to 6) or ts13 (lanes 7 to 18) nuclear extracts were performed at the indicated temperatures in vitro in the absence (−) or presence (+) of Gal4VP16 (lanes 1 to 12) or Sp1 (lanes 13 to 18) and subjected to primer extension followed by gel electrophoresis in 8% denaturing polyacrylamide and autoradiography. (**B**) Quantitation. The results shown in (A) were analyzed and quantitated with the Molecular Dynamics PhosphorImager (University of California, Berkeley). The relative intensities of bands representing basal and Gal4VP16- or Sp1-mediated transcriptional activation in the indicated extracts are plotted as a function of reaction temperature.

ts13 cells. These extracts regained the ability to be activated at 30°C in vitro (Fig. 3). The rescue of the in vitro transcriptional defect by human TFIID suggests that the complex in ts13 cells is indeed altered at the higher temperature and can be complemented by purified human TFIID in vitro.

Next, we attempted to determine whether the defect in the TFIID complex in ts13 extracts is attributable to a mutation in $TAF_{II}250$. It is not possible to restore transcriptional activation by the addition of purified human $TAF_{II}250$ directly to ts13 extracts because the subunits of TFIID do not freely exchange. However, our previous observation that recombinant human $TAF_{II}250$ transiently expressed in HeLa cells was efficiently incorporated into the endogenous complex (*2*) prompted us to perform the following complementation experiment. Cells were transfected with an expression vector encoding either human $TAF_{II}250$ or a control gene, the glucocorticoid receptor (GR), and maintained at either the permissive or nonpermissive temperature (Fig. 4A) (*13*). Whereas neither expression vector had any visible effect at 33.5°C, cells transfected with the human $TAF_{II}250$ expression vector, but not the control GR vector, were able to grow at 39.5°C. Stable ts13 transformant cell lines expressing human $TAF_{II}250$ were selected by their ability to grow at 39.5°C. Nuclear extracts from one such cell line, ts13R3, shown to express human $TAF_{II}250$ by protein immunoblot analysis (Fig. 4B), were prepared and assayed for in vitro transcription. Transcriptional activation by Gal4VP16 was restored and found to be temperature independent in these extracts (Fig. 4C). Similar results were obtained with the transcriptional activator Sp1 (*14*). Therefore, human $TAF_{II}250$ can complement the ts13 cell cycle defect in vivo and restore transcriptional activation in vitro. Recent characterization of another ts mutant cell line, BN462 (*15*), which falls in the same complementation group as ts13, revealed that its cell cycle phenotype is due to a single amino acid change in CCG1 (*16*). This finding suggests that lesions within the hamster CCG1 gene are responsible for the cell cycle arrest and taken together with our results confirms the hypothesis that the transcriptional properties of ts13 are altered as a result of a mutation in $TAF_{II}250$, the largest subunit of TFIID.

A question that remains to be answered is how a mutation in $TAF_{II}250$-CCG1 causes cells to arrest in G_1. It was originally postulated that CCG1 is a promoter-specific transcription factor responsible for regulating genes governing progression from G_1 to S (*7*). Instead CCG1-$TAF_{II}250$ turns out to be a component of the general transcriptional machinery, specifically TFIID (*2*, *3*). However, in ts13 cells, the expression of a subset of genes appears to be specifically down-regulated, including the cell cycle gene cyclin A, whereas the bulk of RNA synthesis is unaffected (*11*, *16*). To test the idea that genes may be differentially affected by the ts13 mutation, we constructed reporter plasmids containing the cyclin A and c-fos promoters for use in transient transfection assays to examine the activity of these promoters at the permissive and nonpermissive temperatures (Fig. 5A). We observed that expression from the cyclin A but not the c-fos promoter decreased markedly when ts13 cells were shifted from 33.5°C to 39.5°C. In contrast, no temperature-sensitive decrease in promoter activity was detected when the cyclin A and c-fos constructs were tested in the ts13 transformant (ts13R3) rescued by the expression of wild-type human $TAF_{II}250$. Taken together these data strongly suggest that in ts13 cells, the temperature-dependent transcriptional defect as a result of the mutation in $TAF_{II}250$ exhibits promoter selectivity. Sequence analysis of the regulatory regions of the cyclin A and c-fos promoters reveals that cyclin A contains consensus binding sites for multiple sequence-specific activators, including Sp1, which are absent from the c-fos promoter region and vice versa (Fig. 5B). Therefore, it appears that only a subset of transcriptional activators is sensitive to the mutation in $TAF_{II}250$. It remains to be determined whether Sp1 is critical for the regulated expression of cyclin A. It is possible that other as yet unidentified factors are required in addition to Sp1 for cyclin A promoter activity.

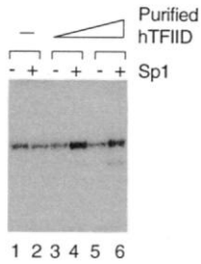


Fig. 3. Wild-type human TFIID can complement the transcriptional defect of ts13 nuclear extracts in vitro. Increasing amounts of immunoaffinity-purifiedhuman TFIID as indicated were added to transcription reactions carried out in 80 μg of ts13 nuclear extracts supplemented with either no activator (lanes 1, 3, and 5) or Sp1 (lanes 2, 4, and 6). Reaction mixtures containing 200 ng of the DNA template GCE4T were incubated at 30°C followed by primer extension analysis, 8% denaturing polyacrylamide gel electrophoresis, and autoradiography (*27*).

In this study, we have observed that transcriptional activation in ts13 nuclear extracts recapitulates the temperature-sensitive phenotype observed in vivo. By immunoprecipitation (*14*), the TFIID complex from ts13 cells appears intact and exhibits no obvious loss of subunits or other gross changes. Our results therefore suggest that the lack of activation most likely reflects a temperature-dependent conformational change induced at the nonpermissive temperature, as opposed to proteolysis of the mutant protein. Therefore, the mutation appears to elicit a subtle change that may alter the interaction of $TAF_{II}250$ with other TAFs known to directly contact this

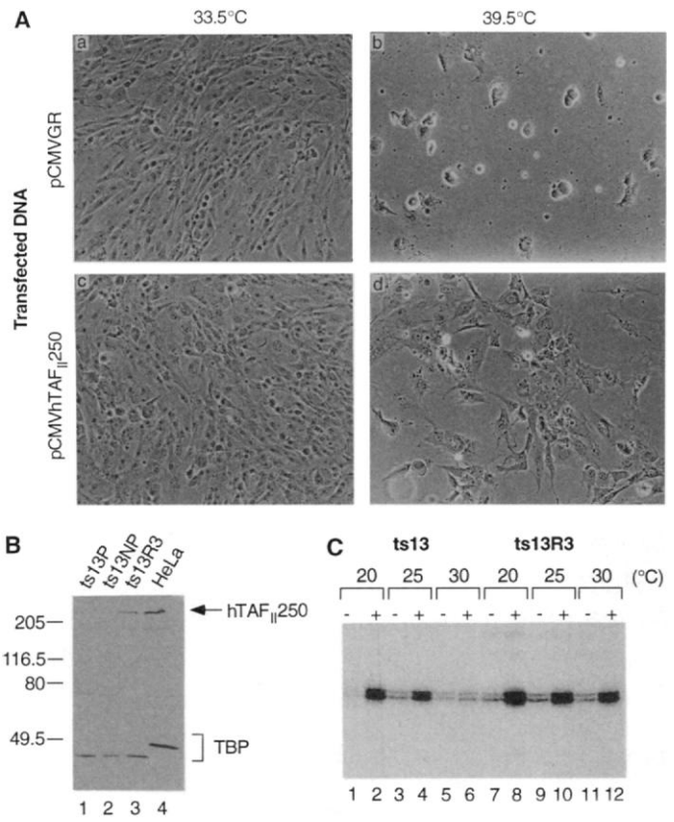
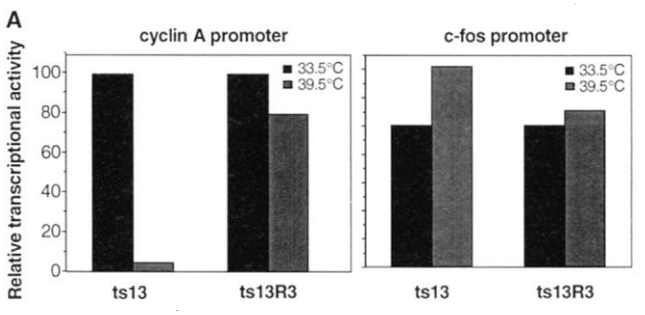


Fig. 4. Recombinant hTAF$_{II}$250 can rescue the temperature-sensitive phenotype of ts13 cells in vivo and in vitro. (**A**) Expression vectors encoding recombinant human TAF$_{II}$250 (pCMVhTAF$_{II}$250) or a control gene, the glucocorticoid receptor (pCMVGR), were introduced into ts13 cells by calcium phosphate precipitation. Transfected cells were then maintained at either 33.5°C (a and c) or 39.5°C (b and d). Cells after 4 days of growth at 33.5°C are shown in panels (a) and (c). Panels (b) and (d) represent ts13 cells transfected with either TAF$_{II}$250 or GR, respectively, which were shifted to 39.5°C for 7 days. (**B**) Stable ts13 transformant ts13R3 expresses hTAF$_{II}$250. Nuclear extracts from ts13 cells grown at 33.5°C (lane 1), at 39.5°C (lane 2), from stable ts13 transformant ts13R3 (lane 3), and from HeLa cells (lane 4) were subjected to 8% SDS–polyacrylamide gel electrophoresis and protein immunoblot analysis. The filter was first incubated with a human antiserum to TAF$_{II}$250 (α-GST-λH1) directed against the COOH-terminal portion of the protein (*2*) and subsequently with a human TATA-binding protein (TBP) monoclonal antibody, SL33 (*28*), which cross-reacts with hamster TBP, to demonstrate that comparable amounts of protein were loaded in each lane. We observed that TBP in hamster cells is smaller than the human homolog. The positions of hTAF$_{II}$250 and of human and hamster TBP are shown on the right. Molecular size standards (in kilodaltons) are shown on the left. (**C**) The hTAF$_{II}$250 subunit restores transcriptional activation in ts13R3 nuclear extracts in vitro. In vitro transcription assays containing 10 ng of G$_5$E4T and 200 ng of pBS+ were carried out in nuclear extracts from ts13 cells (lanes 1 to 6) or ts13R3 cells (lanes 7 to 12). Reaction mixtures were incubated at the indicated temperatures in the absence (−) or presence (+) of Gal4VP16 and subjected to primer extension analysis, gel electrophoresis, and autoradiography.

largest subunit (*17, 18*) and induce the formation of a functionally impaired TFIID complex. Components of TFIID, namely dTAF$_{II}$110 (*19*) and more recently dTAF$_{II}$40 (*20*), have been shown to interact directly with the transcriptional activators Sp1 and Gal4VP16, respectively. Thus, at the nonpermissive temperature, some gene-specific regulatory proteins may be unable to interact productively with the mutant TFIID complex present in ts13 cells. Alternatively, TAF$_{II}$250-CCG1 could produce its promoter-specific effect by directly or indirectly influencing the interaction of TFIID with specific DNA sequences, some of which may include regulatory elements associated with cell cycle genes. Cyclin A has been shown to be important for the progression of mammalian cells from G$_1$ into S phase and the onset of DNA replication (*21*). Our finding that the activity of the cyclin A promoter is specifically impaired in ts13 cells at the nonpermissive temperature further supports the hypothesis that the cell cycle phenotype of ts13 cells is due to promoter-specific transcriptional defects in these cells. Currently, transcription factors that may play a role in DNA replication or the cell cycle include sequence-specific DNA-binding factors, such as BPV E1 and E2 (*22, 23*), human CTF-NF-1 (*24*), and the RB-E2F-cyclin complex (*25*). Here we document that a general transcription factor, the TAF-TBP complex, may also selectively influence the expression of genes responsible for cell cycle progression. The availability of recombinant TAFs and the prospect of reconstituting fully functional complexes may eventually allow us to unravel this unexpected link between transcription factors, TAFs, and cell cycle regulation.



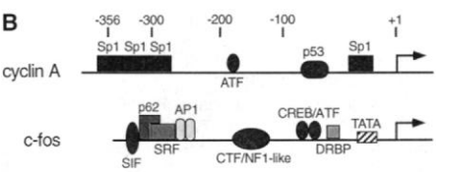


Fig. 5. The transcriptional defect in ts13 cells exhibits promoter selectivity. (**A**) The reporter constructs used in the transient transfection assays include the human cyclin A promoter (−1056 to −41) fused to the luciferase gene and the mouse c-fos promoter (−356 to +109) fused to the chloramphenicol acetyltransferase (CAT) gene (*29*). The ts13 and ts13R3 cells (2 × 10^5 to 5 × 10^5 cells per 10-cm dish) were transfected with 2 μg of the indicated reporter construct, 2 μg of the β-galactosidase gene, and 16 μg of pUC118 by calcium phosphate precipitation. The cells were maintained at either 33.5°C or 39.5°C and harvested after 36 to 48 hours. β-Galactosidase activity was determined as described (*30*) and used to normalize for transfection efficiency. Luciferase activity was measured with the luciferase assay system (Promega) and CAT expression determined according to published procedures (*31*). 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 data represent one experiment performed in duplicate whose results have been reproduced in three independent transfections. Similar results have been obtained with a c-fos luciferase reporter construct that was less active in both cell lines at the nonpermissive temperature. (**B**) Regulatory region of human cyclin A and mouse c-fos promoters. A schematic diagram of the human cyclin A promoter indicating the position of consensus binding sites for the transcriptional activator Sp1, activating transcription factor (ATF), and the tumor suppressor p53 are indicated. The map of the c-fos promoter and the proteins shown to interact with this region have been previously described (*32*).

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12. For the preparation of nuclear extracts, ts13 cells were synchronized by serum starvation and subsequently induced with 10% fetal bovine serum at the permissive (33.5°C) or nonpermissive (39.5°C) temperature as described (*8*). After 20 to 24 hours of growth, nuclear extracts were prepared according to the method of Dignam *et al.* [J. D. Dignam, R. M. Lebovitz, R. G. Roeder, *Nucleic Acids Res.* 11, 1475 (1983)]. Nuclear extracts were also prepared from BHK-21 cells grown at 37°C.
13. The pCMVGR vector contains rat GR under the control of the cytomegalovirus promoter-enhancer [C. M. Gorman, D. Gies, G. McCray, M. Huang, *Virology* 171, 377 (1989)]. The pCMVhTAF$_{II}$250 vector was constructed by replacement of the coding region of rat GR in pCMVGR with that of hTAF$_{II}$250. The ts13 cells plated on 6-cm dishes were transfected in duplicate with either 12 μg of pCMVGR or 20 μg of pCMVhTAF$_{II}$250 by calcium phosphate precipitation. After 16 hours at 33.5°C, half of the cells were shifted to 39.5°C. After 4 days at 33.5°C or 7 days at 39.5°C, photomicrographs were taken at ×125 magnification.
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27. Transcription assays were done essentially as described [B. F. Pugh and R. Tjian, *Cell* **61**, 1187 (1990)]. Reaction mixtures (25 μl) containing 10-ng template DNA, 50 to 100 μg of nuclear extract, 200 ng of nonspecific DNA (pBS+), and either 50 ng of Gal4VP16 or 100 ng of Sp1 where indicated, were preincubated for 30 min at the indicated temperature in the absence of ribonucleoside triphosphates (NTPs). After the addition of NTPs to a final concentration of 0.5 mM, the reaction was allowed to proceed for an additional 10 to 15 min. The products were detected by primer extension, followed by denaturing polyacrylamide gel electrophoresis, and visualized by autoradiography. The DNA template containing five Gal4-binding sites upstream of the adenovirus E4 promoter (G_5E4T) has been described [Y.-S. Lin, M. F. Carey, M. Ptashne, M. R. Green, *Cell* **54**, 659 (1988)]. GCE4T was constructed by insertion of the six GC boxes from SV40 upstream of the adenovirus E4 promoter in the E4T template.
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33. We are indebted to C. Basilico for providing the ts13 cell line and B. Johnson for technical assistance in tissue culture. We thank J. Goodrich for providing purified Gal4VP16, J.-L. Chen for purified Sp1, N. Tanese for 1.0 M KCl fraction of LTRα3 cells, G. Gill for the GCE4T and G_5E4T DNA templates, Genentech for the use of the pCMVGR expression vector, S. Ruppert for constructing pCMVhTAF$_{II}$250, T. Nikaido for the human cyclin A promoter, and M. Gilman for the mouse c-fos promoter. The SL33 monoclonal antibody and the antibody to hTAF$_{II}$250 peptide were provided by N. Hernandez and T. Sekiguchi, respectively. We also thank the lab of D. Rio for help with and use of their photomicrographic apparatus. We especially thank J. Goodrich, S. Ruppert, G. Gill, J.-L. Chen, and members of the Tjian lab for valuable discussions and for critically reading the manuscript. E.H.W. was supported by a postdoctoral fellowship from the American Cancer Society (grant PF-3686). Funded in part by a grant from the NIH to R.T.

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Mapping the Lectin-Like Activity of Tumor Necrosis Factor

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Tumor necrosis factor (TNF), but not lymphotoxin (LT), is directly trypanolytic for salivarian trypanosomes. This activity was not blocked by soluble 55-kilodalton and 75-kilodalton TNF receptors, but was potently inhibited by *N,N'*-diacetylchitobiose, an oligosaccharide that binds TNF. Comparative sequence analysis of TNF and LT localized the trypanocidal region, and synthetic peptides were trypanolytic. TNF molecules in which the trypanocidal region was mutated or deleted retained tumoricidal activity. Thus, trypanosome-TNF interactions occur via a TNF domain, probably with lectin-like affinity, which is functionally and spatially distinct from the mammalian TNF receptor binding sites.

The pleiotropic cytokine TNF has numerous effects on mammalian cells, initiated by binding to high-affinity receptors (*1*). In addition to binding to mammalian 55-kD and 75-kD receptors, TNF has a lectin-like specificity (*2*), the physiological relevance of which remains to be determined. TNF interacts directly with certain parasites (*3, 4*) as well as bacteria (*5*): In the case of the pathogenic bacteria *Shigella flexneri*, the interaction does not involve molecules comparable to the classical mammalian TNF receptors, implying the existence of an alternative recognition domain of TNF for bacterial components. The lectin-like activity of TNF represents a possible candidate for such an alternative recognition, since it is completely dissociable from the tumoricidal activity (*2*).

This study defines the region of TNF that interacts with salivarian trypanosomes. TNF inhibits the development of *Trypanosoma brucei brucei* in vivo (*6*) and in vitro: Both mouse and human TNF (m/hTNF), but not human lymphotoxin (hLT), are trypanolytic for purified bloodstream forms of *T. brucei brucei* (Fig. 1A). Preincubation of mTNF with soluble extract of *T. brucei brucei* potently inhibited the trypanolytic activity (Fig. 1A) without affecting the tumoricidal activity on L929 cells (Fig. 1B). These results indicate that mTNF interacts with *T. brucei brucei* and that this interaction does not interfere with the binding of mTNF to the mouse 55-kD TNF receptor, which mediates the bioactivity of m/hTNF on L929 cells (*7*).

To further substantiate the possibility that the mTNF binding site for the 55-kD TNF receptor is not involved in trypanocidal activity, we performed inhibition experiments with a soluble form of the human 55-kD TNF receptor (shTNF-RI), which binds both hTNF and mTNF (*8*). Preincubation of mTNF with shTNF-RI resulted in a total inhibition of the mTNF tumoricidal activity on L929 cells, but only marginal inhibition of the trypanocidal activity on *T. brucei brucei* (Table 1). Similar results were obtained for a soluble form of the

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