TAFII250, a component of the general transcription factor, TFIID, is required for the transcription of a subset of genes, including those involved in regulating cell cycle progression. The tsBN462 cell line, with a temperature-sensitive mutation of TAFII250, grows normally at 32 °C, but when grown at 39.5 °C, it differentially arrests transcription of many, but not all, genes. The present studies examine the basis for the requirement for TAFII250. We show that the basal promoter of a major histocompatibility complex class I gene requires TAFII250. This dependence can be overcome by select upstream regulatory elements but not by basal promoter elements. Thus, the coactivator CIITA rescues the basal promoter from the requirement for TAFII250, whereas introduction of a canonical TATAA box does not. Similarly, the SV40 basal promoter is shown to require TAFII250, and the presence of the 72-base pair enhancer overcomes this requirement. Furthermore, the SV40 72-base pair enhancer when placed upstream of the basal class I promoter renders it independent of TAFII250. These data suggest that the assembly of transcription initiation complexes is dynamic and can be modulated by specific transcription factors.

Transcription of eukaryotic genes is initiated by the orderly assembly of a series of general transcription factors (1). The general transcription factor, TFIID, nucleates the assembly through the binding of its component TBP to a TATAA box or other appropriate promoter element. Other components of TFIID, the TBP-associated factors (TAFs),1 recruit TFIIF1 and TFIIFB, which serves as a bridge to the RNA polymerase II complex, and TFIIE, -F, and -H. As many as 12 distinct TAFs have been isolated and their functions are beginning to be characterized (1). Through their interactions with upstream transcription factors, the TAFs also function as coactivators. In Drosophila, the TAFs appear to play a role in promoter selectivity and are required to initiate at promoters that lack a TBP binding site (2).

Recent evidence in yeast and mammalian cells indicates that not all TAFs are essential for the transcription of all genes (3, 4). For example, inactivation of yeast TAF130, the homolog of TAFII250, affects only a subset of genes. Similarly, a point mutation of TAFII250 results in a temperature-sensitive cell line, tsBN462. At the restrictive temperature, cell cycle is arrested at G1, and transcription of a set of cyclin genes is terminated (5–7). However, other genes such as c-fos and c-myc continue to be transcribed at the restrictive temperature (5–7).

Given the disparity in the requirements of different promoters for TAFII250, it is of interest to determine whether the primary role of TAFII250 is to function as a coactivator of upstream transcription factors or as a general transcription factor component that is necessary for assembling a preinitiation complex. Various studies have suggested that promoter dependence on TAFII250 is determined by upstream elements (7). Thus, TAFII250-dependent transcription of the cyclin A gene was mapped to an ATF binding site in the extended cyclin A promoter (6). Similarly, an analysis of the cyclin D1 promoter concluded that the TAFII250 mutation in the ts cells affects the function of upstream activators, not the basal promoter (7).

Together, these studies suggest that the primary role of TAFII250 is a coactivator of upstream activators. However, at least for the cyclin D1 promoter, a function of TAFII250 at the core promoter has not been excluded. Furthermore, studies of both human cyclin A and several yeast promoters have shown that the requirement for TAFII250 is determined by the basal promoter; it has been suggested that TAFII250 is required for promoters that do not contain canonical TATAA boxes (7, 8).

The present studies were designed to assess the role(s) of TAFII250 in regulating major histocompatibility complex (MHC) class I expression. Unlike previously studied promoters, the promoters of genes encoding MHC class I are ubiquitously expressed and do not regulate cell cycle or growth. The MHC class I genes encode cell surface molecules that, as dimers with β2-microglobulin, serve as receptors for viral peptides, thereby triggering a cellular immune response. Expression of the class I genes and β2-microglobulin genes are coordinately regulated in vivo. Consistent with their role in immune surveillance against intracellular pathogens, the class I and β2-microglobulin genes are expressed in nearly all somatic cells (9). However, the level of expression displays tissue-specific variation, which is further affected by hormonal signals. Although a complex array of upstream DNA elements modulates class I expression, the basal promoter itself is constitutively active in the absence of any upstream activation (10, 11). The upstream elements function primarily to modulate this basal level, resulting in either increases or decreases, depending on the tissue and hormonal milieu.

We have previously reported that expression of the MHC class I gene, PD1, depends upon a functional TAFII250 (12). In particular, impairment of TAFII250 function, either through inactivation in the tsBN462 cell line or through inactivation of the intrinsic histone acetyltransferase activity, prevented tran-

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‡ The abbreviations used are: TAF, TBP-associated factor; MHC, major histocompatibility complex; bp, base pair(s); CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; LTR, long terminal repeat; CRE, cAMP-responsive element; CREB, cAMP-responsive element-binding protein; NFκB, nuclear factor κB; CBP, CREB-binding protein. AdML, adenovirus major late promoter.
TAFII250, in support of basal class I expression, is coactivator capable of recruiting appropriate coactivators. Furthermore, requires the presence of specific upstream regulatory elements for activity, can effectively replace TAFII250 function. In contrast, the requirement for TAFII250 can be overcome by select transcription factors acting through upstream regulatory elements. In particular, the coactivator CIITA, which activates class I promoter activity, can effectively replace TAFII250 function. In contrast, USF1 and USF2, which also activate the class I promoter, do not overcome the TAFII250 requirement.

We further show that the SV40 basal promoter is similarly dependent on TAFII250. This dependence is overcome by the intact viral 72-bp enhancer and by the isolated octamer and AP1 subelements of the enhancer. Interestingly, these viral enhancer elements can overcome the TAFII250 dependence of the class I promoter when introduced upstream.

We conclude that TAFII250-independent transcription requires the presence of specific upstream regulatory elements capable of recruiting appropriate coactivators. Furthermore, this leads to the suggestion that an important activity of TAFII250, in support of basal class I expression, is as a coactivator. Taken together, the data suggest that the assembly of transcription initiation complexes is a dynamic process that can be modulated by specific transcription factors.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—The tsBN462 cells, containing a point mutation in the TAFII250/CCG1 gene and derived from BHK cells as described (13, 14) were obtained from T. Sekiguchi, Salk Institute. The cells were maintained at 32 °C, 7.5% CO₂ in Dulbecco’s modified Eagle’s medium (Biolo) with 10% fetal calf serum. Transfections were done by CaOPO₄, as described previously, using 5 µg of DNA, unless otherwise indicated (12). Following transfection, cells were incubated at 32 °C for 24 h, after which time they were refed with fresh medium and either shifted to 39.5 °C or left at 32 °C. After an additional 24 h, cells were harvested and assayed for CAT activity, as described previously (11). All activities were corrected for protein concentration.

**DNA Constructs**—TAFII250 expression plasmid was a gift of R. Tjian (University of California, Berkeley, CA). The HIVLTR was pBennCAT (15). Rous sarcoma virus, murine leukemia virus, and AdML promoter constructs were as described previously (12, 16). The PD1 deletion constructs and promoter mutations were described previously (11, 17). The β₂-microglobulin promoter construct contained 209 bp of upstream sequence (18). The HLA-E promoter construct contained 155 bp of upstream sequence and was derived from the HLA 6.2 genomic clone (19) by polymerase chain reaction and cloning into the HindIII/BamHI sites of the pSV3CAT vector. The enhancer/promoter SV40 constructs were as follows, pSV2, the enhancer/promoter, and pSV3, the basal promoter construct, were as described (20, 21). The SV40 enhancer subelement constructs were generated by replacing SV40 sequences between the NdeI and Spal sites of pSV3CAT with synthetic oligonucleotides, as described previously (11). The 72-bp enhancer of the SV40 enhancer/promoter was isolated by digestion and introduced into the XbaI site of the class I promoter construct (11). The β₂-microglobulin construct was ligated to the CAT reporter gene; the HIVLTR was also ligated to luciferase. The CIITA expression plasmid was provided by Dr. Jenny Ting, University of North Carolina (22).

**In Vitro Transcription**—Nuclear extracts were prepared from tsBN462 cells and grown at either 32 °C or 39.5 °C, according to the protocol of Dignam et al. (23). In vitro transcription reactions contained 135 µg of nuclear extract, 5 µg of −313 DNA, or 1 µg of cytomegalovirus promoter RNA. Each of the promoters was ligated to a CAT reporter and transfected into tsBN462 cells at the permissive temperature (32 °C). After 24 h, cells were either shifted to the nonpermissive (39.5 °C) temperature or left at the permissive temperature for an additional 24 h prior to harvesting. All experiments were repeated at least three times, each in triplicate. To ensure that the observed effects were reporter independent, the HIV LTR was tested both driving a CAT reporter and a luciferase reporter; no difference in the response was observed. MHC class Ia, classical class I gene, PD1, with 313 bp of upstream sequences and no canonical TATAA box (−313CAT); MHC class IIb, nonclassical class I gene, HLA-E, with 80 bp of upstream sequences and a canonical TATAA box; β₂-microglobulin with 206 bp of upstream sequence and a canonical TATAA box.

| Promoter | Relative activity (39.5 °C/32 °C) | Canonical TATAA box |
|----------|----------------------------------|---------------------|
| MHC class Ia, PD1 | 0.24 ± 0.03 | No |
| MHC class IIb, HLA-E | 0.42 ± 0.02 | Yes |
| β₂-microglobulin | 0.33 ± 0.02 | Yes |
| Murine leukemia virus | 0.99 ± 0.06 | |
| SV40 | 1.7 ± 0.16 | |
| Rous sarcoma virus | 3.9 ± 0.44 | |
| HIV LTR | 3.8 ± 0.40 | |
| AdML | 3.6 ± 0.1 | |

(Promega) DNA and were performed at 20 °C. Analysis of product was by primer extension (12).

**RESULTS**

**Differential Sensitivity to a TAFII250 Mutation among Different Promoters**—A series of cellular and viral promoters was examined for their dependence on TAFII250. We tested three ubiquitously expressed and related cellular promoters whose products are involved in regulating immune responses: an MHC class Ia promoter, PD1, whose gene encodes a classical transplantation antigen; an MHC class IIb promoter, HLA-E, which governs expression of a nonclassical class I molecule; and the promoter of the β₂-microglobulin gene, which encodes the light chain that associates with both of the class I heavy chains. All three promoter constructs consisted of their basal promoter and upstream regulatory elements. As shown in Table I, PD1 promoter activity in tsBN462 cells is markedly reduced at the restrictive temperature, indicating that it depends on functional TAFII250. The reduction in PD1 promoter activity is specific to the tsBN462 mutation; in the parental BHK cells, the PD1 promoter construct −313CAT is 1.8 ± 0.5 times more active at 39.5 °C than at 32 °C. As shown in Fig. 1, cotransfection of wild type TAFII250 with the class I promoter construct restored promoter activity in tsBN462 cells at the restrictive temperature. From this, we conclude that the class I promoter depends upon TAFII250. Both the β₂-microglobulin promoter and the HLA-E promoter are similarly dependent on TAFII250. Thus, among the three related promoters, all require TAFII250.

It has been suggested that TAFII250 dependence correlates with the absence of a canonical TATAA box (7, 8). Consistent with this suggestion, the PD1 promoter does not contain a canonical TATAA box and depends on TAFII250. In contrast, the promoters of both the β₂-microglobulin and HLA-E genes contain a canonical TATAA box but still require functional TAFII250 for activity (Table I). Thus, there is no direct correlation between the requirement for TAFII250 and the presence of a canonical TATAA box in these cellular promoters.

Five viral promoter/enhancer constructs were also examined (murine leukemia virus, SV40, Rous sarcoma virus, AdML, and HIVLTR); all were as active, or more active, in the tsBN462 cells at the restrictive temperature as at the permissive temperature (Table I). The findings with the SV40 promoter are
is to arrest cells at G1 in the cell cycle. Thus, in the above experiments, cells were cotransfected with the class I promoter construct, −313CAT, and increasing amounts of a TAFII250 expression vector. Cells were incubated for 24 h at the permissive temperature, 32 °C, after which time they were either shifted to the restrictive temperature, 39.5 °C, or left at 32 °C for an additional 24 h before harvesting and assaying for CAT activity. CAT activity is stable for at least 24 h at 39.5 °C in these cells (data not shown). The results are presented as promoter activity at the restrictive temperature, relative to the permissive, at each TAFII250 concentration.

Consistent with those previously reported by others (5), to ensure that these results were not because of the use of the common CAT reporter, the HIVLTR was tested with a luciferase reporter, with the same outcome (data not shown). Thus, the viral promoters do not require a functional TAFII250. The remaining studies were directed at characterizing the basis of the PD1 promoter dependence on TAFII250.

In Vitro Transcription of the Class I Promoter Requires a Functional TAFII250—One of the effects of shifting the tsBN462 cells to 39.5 °C, in addition to inactivating TAFII250, is to arrest cells at G1 in the cell cycle. Thus, in the above in vivo transfection experiments, it is not possible to distinguish whether the temperature shift directly affects the basal promoter requirement for TAFII250 or reduces transcription indirectly through cell cycle arrest. Indeed, previous studies have demonstrated that the rate of transcription of class I genes is cell cycle linked. To determine whether inactivation of TAFII250 directly affects transcription of class I, the ability of nuclear extracts from tsBN462 cells to direct in vitro transcription of the promoter was tested. Extracts were derived from cells grown either at 32 °C or at 39.5 °C and were used to direct transcription from the −313CAT class I promoter construct. Extracts derived from cells grown at 32 °C were fully competent to direct transcription from the class I promoter (Fig. 2, left). However, extracts derived from cells grown at 39.5 °C were defective in transcribing the same promoter (Fig. 2, left). In striking contrast, in vitro transcription of the cytomegalovirus promoter was increased in extracts prepared from cells grown at 39.5 °C, relative to that prepared from cells grown at 32 °C (Fig. 2, right). Because inhibition of class I promoter activity is observed in a cell-free system, it is not secondary to the arrest at G1 of cells following the shift to 39.5 °C.

Dependence on TAFII250 Maps to the Basal Promoter of PD1—Because the cellular promoter constructs tested contained upstream activating sequences, the observed differential sensitivity among the promoters to the TAFII250 mutation in the tsBN462 cells could be either a reflection of differences in those upstream sequences or of differences in basal promoter architecture. To address this question, the dependence on TAFII250 of a series of nested 5′-truncations of the PD1 promoter was examined. The longest promoter construct, −1013, contains all of the upstream regulatory elements necessary to determine normal patterns of expression of the gene, as demonstrated in transgenic mice (10). Deletion of upstream sequences between −1013 and −516 bp removes a series of silencer elements and a complex regulatory element that governs tissue-specific levels of expression (24) (Fig. 3). Further truncations successively remove additional tissue-specific regulatory elements (−416), an E-box that is the target for USF activation (−313), enhancer A, the interferon-response element, and the cAMP-responsive element (CRE) (−68) (16, 25, 26). The −68 promoter construct constitutes the basal promoter and contains only a CCAAT box, a noncanonical TATA box, an Inr, and an S-box important for promoter function (11). Successive truncations between −1013 and −416 increase promoter activity (Fig. 3). It is important to note that, unlike most basal promoters, the −68 promoter construct is constitutively active and does not require upstream enhancers (Fig. 3).

The full-length −1013-bp promoter construct is active in the tsBN462 cells at the permissive temperature but is inhibited at the restrictive temperature (Fig. 3). Each of the successive PD1 truncations from −1013 bp increases the promoter dependence on TAFII250 such that the −68-bp construct is approximately 3-fold more sensitive than the −1013-bp construct, despite the fact that at 32 °C, it is significantly more active than the −1013 bp construct (Fig. 3). Thus, although the extended −1013 promoter is still sensitive to partial inhibition at 39.5 °C, its upstream sequences confer a significant level of protection on the basal promoter.

In the pattern of its requirement for TAFII250, the PD1 promoter differs from the cyclin A promoter or the cyclin D1 promoter (6, 7). In the former case, an upstream ATF/CREB binding site has been reported to function as a temperature-sensitive response element that confers a dependence on TAFII250 (6). Like the cyclin A promoter, the PD1 promoter contains a functional CRE that has been demonstrated to bind and be activated by ATF/CREB family members (27). However, unlike the cyclin A promoter, the presence of the CRE does not

![Graph: Exogenous TAFII250 restores class I promoter activity in tsBN462 cells at the restrictive temperature.](http://www.jbc.org/content/271/8/10162/F2.large.jpg)

**Fig. 2. In vitro transcription of the class I promoter depends upon a functional TAFII250.** In vitro transcription assays on the class I promoter construct, −313 (left) or the cytomegalovirus promoter construct (right) were performed with extracts prepared from tsBN462 cells grown at either 32 °C or 39.5 °C. The class I-specific transcript is 67 bp, whereas the cytomegalovirus transcript is 75 bp. The experimental lanes were derived from different parts of the gel and have been aligned for the purposes of the figure.

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2 J. D. Weissman and D. S. Singer, unpublished observations.
increase the PD1 promoter dependence on TAF\textsubscript{II}250 (compare -313-bp and -68-bp constructs). Thus, these findings also indicate that ATF/CREB is not a general determinant of TAF\textsubscript{II}250 dependence.

### Basal Promoter Elements Do Not Establish a Requirement for TAF\textsubscript{II}250

The above studies map TAF\textsubscript{II}250 dependence to the PD1 basal promoter, raising the question of whether discrete basal promoter elements establish this requirement. A series of promoter mutations were introduced into the PD1 basal promoter. In one, the noncanonical TATAA box, TCTAA, was restored to a canonical TATAA box (TCT>TATAAA); in another, it was mutated further away from the canonical sequence (TATA M1). Both mutant promoters maintained the requirement for a functional TAF\textsubscript{II}250 (Fig. 4). Thus, the presence of a TATAA box is not sufficient to overcome the requirement for TAF\textsubscript{II}250. Similarly, the complete absence of a TATAA box does not increase the dependence. A third mutant, in which the Inr was mutated (M3), also retained its dependence on TAF\textsubscript{II}250 (Fig. 4). Interestingly, a mutant construct in which both the TCTAA box and Inr were mutated was active and remained dependent on TAF\textsubscript{II}250 (Fig. 4) (11). All of these promoter mutants retained significant activity, both in the tsBN462 cells at the permissive temperature (Fig. 4) and in HeLa cells (11). An extended mutation of the entire central region between the TCTAA and Inr renders the promoter inactive (8); it is inactive at both the permissive and restrictive temperatures in tsBN462 cells. In summary, the class I TCTAA box and the Inr are neither necessary nor sufficient for transcription initiation. Further, no specific promoter element was identified that confers a dependence on TAF\textsubscript{II}250.

### Upstream Enhancers Can Confer Independence of TAF\textsubscript{II}250 on Basal Promoters

The role of upstream enhancers in relieving the basal promoter of its dependence on TAF\textsubscript{II}250 was examined for the SV40 enhancer/promoter construct whose 72-bp repeat enhancer has been extensively characterized. Removal of the two 72-bp enhancer elements (leaving the 21-bp Sp1-binding GC repeats in place) significantly reduces SV40 promoter activity; further removal of the 21-bp repeats eliminates all promoter activity. In the absence of the 72-bp enhancers, the activity of the SV40 promoter dropped 2-fold in the tsBN462 cells at the restrictive temperature relative to the permissive temperature, unlike the SV40 enhancer/promoter construct which was activated by 2-fold (Fig. 5). Thus, removal of the 72-bp enhancer markedly alters the promoter sensitivity to functional TAF\textsubscript{II}250. These data are consistent with the interpretation that the SV40 basal promoter is inherently dependent upon TAF\textsubscript{II}250 but that the presence of the enhancer elements overcomes this dependence. The ability of the enhancers to alleviate TAF\textsubscript{II}250 dependence could be due either to a single subelement within the enhancer or to the cumulative effects of multiple enhancers. To distinguish between these possibilities, the ability of a single enhancer and isolated subelements to alter the TAF\textsubscript{II}250 dependence of the SV40 basal promoter was determined. As shown in Fig. 5, the introduction of a single copy of the 72-bp enhancer element was sufficient to completely protect the SV40 basal promoter from inhibition at the restrictive temperature in tsBN462 cells. Furthermore, the individual octamer or AP1 subelements were almost as effective as the whole 72-bp element in restoring independence of TAF\textsubscript{II}250 to the SV40 basal promoter.

However, not all enhancer elements are capable of altering basal promoter requirements. As seen in Fig. 5, the SV40 NF\textsubscript{xB} element does not protect the basal SV40 promoter from its dependence on TAF\textsubscript{II}250. This is true even in cells treated with phorbol ester to activate nuclear translocation of NF\textsubscript{xB} (data not shown). The failure of the NF\textsubscript{xB} element to protect the promoter and the ability of the octamer and AP1 elements to protect do not reflect a differential activation of the promoter by these elements; all three isolated elements activate the promoter to the same extent (data not shown). In addition, the GC-rich 21-bp Sp1 core promoter elements are also incapable of fully protecting the SV40 basal promoter from TAF\textsubscript{II}250 dependence. Because the SV40 promoter is inactive in the absence of these 21-bp elements, it is not possible to determine whether they confer some level of protection on the promoter. Similarly, the central S box of the PD1 promoter, which is a binding site for, and is activated by, Sp1, is dependent on a functional TAF\textsubscript{II}250.

### Coactivators and Activators Can Modulate the Class I Promoter Requirement for TAF\textsubscript{II}250

Because the SV40 enhancer confers TAF\textsubscript{II}250 independence on the basal SV40 promoter, we asked whether it could similarly rescue the heterologous PD1 promoter. A single SV40 72-bp enhancer was placed upstream of either the -68-bp or -313-bp class I PD1 promoter constructs. In the presence of the SV40 enhancer, class I promoter activity was markedly increased. At the same time, the dependence of the class I promoter on TAF\textsubscript{II}250 was significantly reduced (−68 construct) or eliminated (−313 construct).
Although neither the isolated octamer nor AP1 sub-elements was able to fully protect the class I promoter, each did afford partial protection (data not shown). Therefore, the SV40 viral enhancer element is able to alter the downstream promoter sensitivity to functional TAFII250, both on homologous and heterologous basal promoters.

The ability of the heterologous viral enhancer element to overcome the TAF II250 dependence of the class I promoter suggested that endogenous activators might have similar activities. We recently reported that the helix-loop-helix factors, USF1 and USF2, are strong activators of class I transcription in HeLa cells, binding to the upstream E-box element (16). Therefore, we next asked whether either of these activators could rescue the class I promoter from the requirement for TAFII250. As shown in Table II, the class I -416-bp promoter construct, which contains an E-box, is activated by both USF1 and USF2 when co-transfected into tsBN462 cells maintained at 32 °C. The levels of activation are markedly lower than observed in HeLa cells (16). This is not a function of the TAFII250 mutation, because similarly low levels of USF activation are observed in the parental BHK cells at 32 °C (data not shown). No protection of promoter activity was observed by either USF1 or USF2. At 39.5 °C, promoter activity is markedly inhibited even in the presence of either USF1 or USF2 (Table III). USF1 and USF2 were active at both temperatures, as evidenced by the fact that each efficiently activated a construct.
TABLE II

The SV40 72-bp enhancer can partially rescue the class I promoter from dependence on TAFII250

The class I promoter constructs, −68 and −313CAT, in the presence or absence of the SV40 72-bp enhancer, were transfected into tsBN462 cells at 32 °C. After 24 h, the cells were either shifted to 39.5 °C or left at 32 °C, for an additional 24 h before harvesting and assaying for CAT activity. The enhancement is the relative activity of the class I promoter in the presence or absence of the enhancer, as measured at 32 °C. The relative activity is the ratio of activity of a given promoter at the two temperatures. Results are from representative experiments, each performed in triplicate.

| Promoter construct | Fold enhancement (+enhancer−enhancer) | Relative activity (39.5 °C/32 °C) | P |
|--------------------|----------------------------------------|-----------------------------------|---|
| −68CAT             | 1.0                                    | 0.19 ± 0.05                       | <0.02 |
| −68CAT + enhancer  | 3.1 ± 0.3                              | 0.52 ± 0.07                       | <0.02 |
| −313CAT            | 1.0                                    | 0.49 ± 0.04                       | <0.01 |
| −313CAT + enhancer | 14.6 ± 0.43                            | 1.38 ± 0.18                       | <0.01 |

TABLE III

USF1 and USF2 fail to activate in the absence of a functional TAFII250

The class Ia (−313 and −416CAT) and U2E1b promoters, ligated to the CAT reporter gene, were co-transfected with either an empty expression vector or USF1 or USF2 DNA-containing expression vector into tsBN462 cells at 32 °C. After 24 h, cells were either left at 32 °C or shifted to 39.5 °C for an additional 16 h. The class Ia PD1 promoter, −416CAT, contains 416 bp of upstream sequences; −313CAT contains 313 bp. The U2E1b promoter contains two copies of an E-box element upstream of the AdML promoter.

| Promoter construct | Activator | Relative promoter activity 32 °C | Relative promoter activity 39.5 °C | Relative activity (39.5 °C/32 °C) |
|--------------------|-----------|-------------------------------|-------------------------------|-----------------------------------|
|                    | USF/vector|                               |                               |                                   |
| 416                | Vector    | 1                             | 1                             | 0.55                              |
|                    | USF1      | 1.3                           | 0.6                           | 0.25                              |
|                    | USF2      | 3.4                           | 1.6                           | 0.25                              |
| U2E1b              | Vector    | 1                             | 1                             | 11.9                              |
|                    | USF1      | 3.1                           | 3.6                           | 13.7                              |
|                    | USF2      | 2.9                           | 2.3                           | 9.4                               |

6. Consistent with the finding that CBP/p300 do not regulate class I promoter activity in fibroblasts, neither CBP nor p300 affected the promoter requirement for TAFII250 (data not shown). From these studies we conclude that upstream elements that recruit the appropriate coactivators are capable of modulating the requirement for TAFII250.

DISCUSSION

The requirements for initiation of transcription are still incompletely understood. Although TBP and its associated TAFs clearly play important roles in nucleating the formation of the preinitiation complex, their precise functions appear to differ among different promoters. In the present study, we have examined the factors that contribute to a promoter’s requirement for TAFII250. We have demonstrated that the basal promoter of class I requires TAFII250 but that this requirement does not correlate with the presence of a specific promoter element. Similarly, the activity of the basal SV40 promoter depends on TAFII250. The transcriptional requirement for TAFII250 can be overcome by the presence of select upstream activators; CIITA, but not USF, rescued the class I basal promoter, whereas Oct and Ap1 sites, but not NFκB, rescued the SV40 basal promoter.

The present studies begin to define the basis for promoter requirements for TAFII250. It has been proposed previously that basal promoters lacking a canonical TATAA box require a functional TAFII250 to nucleate the transcription apparatus either directly or indirectly (7, 8). Indeed, the basal promoter of the PD1 promoter does not have a canonical TATAA box and requires functional TAFII250. However, the related HLA-E promoter and the coordinately regulated β₂-microglobulin promoter both have canonical TATA boxes, yet both require TAFII250. Furthermore, mutation of the PD1 basal promoter to introduce a canonical TATAA box does not abrogate the promoter’s dependence on TAFII250. From these findings, we conclude that promoter sequence elements do not determine the requirement for TAFII250.

Promoter dependence on TAFII250 also is unlikely to be a simple reflection of basal promoter strength. The extended class I promoter construct, −1013 CAT, is the least active of the constructs examined. Yet, it is also the least dependent on TAFII250. The dependence of the basal SV40 promoter on TAFII250 is overcome by the placing of either the AP1 or octamer elements upstream; both do increase promoter activity. However, the NFκB element also enhances promoter activity and to the same extent; but it does not affect the basal promoter dependence on TAFII250. In previous studies of the cyclin A promoter, demonstrating that upstream elements conferred dependence on TAFII250, the basal promoter is only minimally active in the absence of upstream elements, so its dependence on TAFII250 is more difficult to assess (6). Nevertheless, even in those studies, residual activity of the cyclin A promoter can be seen to drop 8-fold at the restrictive temperature in the temperature-sensitive cells (6). Taken together, the data indicate that dependence on TAFII250 is unlikely to be a simple reflection of basal promoter strength. Indeed, it is likely that all basal promoters depend on TAFII250, but the magnitude of the dependence varies based on particular upstream elements and the co-factors that they recruit.

The dependence on TAFII250 of both cellular and viral basal promoters can be mitigated by some upstream regulatory elements. For example, the basal class I promoter requires TAFII250, but significant reversal of this dependence is achieved by exogenous upstream sequences or by the introduction of the SV40 72-bp enhancer. Similarly, whereas the basal promoter of SV40 is dependent on TAFII250, the intact enhancer/promoter is not; its activity actually increases at the restrictive temperature. Even the isolated subelements of the
viral enhancer, the octamer and the AP1 binding sites, reduce the promoter's requirement for TAFII250. Finally, modulation of TAFII250 dependence by upstream sequences also has been observed in the cyclin genes (5–8). In one case, removal of upstream sequences from the cyclin A promoter reduced both promoter activity and its requirement for TAFII250.

Not all elements known to regulate the downstream basal promoter affect its dependence on TAFII250. In the present study, we have shown that neither the NFκB element of the SV40 promoter nor the E-box of the class I promoter alters the downstream basal promoter requirements. No obvious sequence features among the regulatory elements have emerged from these studies to explain the differences in their downstream effects.

The observed differences among upstream regulatory elements in modulating dependence on TAFII250 may be determined by the transcription factors that associate with the elements and their different interactions with the preinitiation complex. The ATP/CREB family increases the dependence of the cyclin A promoter on TAFII250 (6). Consistent with that finding, CBP/p300, which are coactivators for ATP/CREB and function through the CRE element, do not overcome class I promoter dependence on TAFII250 (data not shown). However, significant reversal of the class I promoter dependence is achieved by introduction of the CIITA coactivator, which also targets the homologous CRE element.

One model to explain our observations is that activators that function by interacting directly with TAFII250 fail to do so at the restrictive temperature in the tsBN462 cells. On the other hand, activators that do not interact with TAFII250 are able to overcome the dependence on TAFII250, possibly by replacing TAFII250 function or by recruiting other coactivators. For example, CIITA rescues the class I promoter from dependence on TAFII250 and both Oct and AP1 rescue the SV40 promoter. None of these factors is known to interact with TAFII250, although they do interact with other components of TFIID (28–30). In contrast, NFκB is known to interact with TAFII250 (31) and is unable to overcome the requirement for TAFII250 of the SV40 basal promoters. In support of this hypothesis, we have demonstrated that the coactivator, CIITA, contains histone acetyltransferase activity and is able to replace the function of TAFII250 on the class I promoter. This leads to the speculation that CIITA relieves the dependence on TAFII250 of the class I promoter by replacing its histone acetyltransferase activity. However, the presence of histone acetyltransferase activity alone is not sufficient, because CBP/p300 also has histone acetyltransferase activity but does not rescue the promoter. Future experiments, to test this prediction, are directed toward a molecular characterization of CIITA rescue from the TAFII250 requirement.

In conclusion, we have demonstrated that the TAFII250 requirements for transcription initiation not only differ among different promoters, but can be modulated by upstream activators for a given promoter. This observation raises the possibility that different preinitiation complexes initiate transcription of a given promoter, either in different tissues or in a single cell in response to dynamic regulatory signals.

Acknowledgments—We gratefully acknowledge Drs. Susan Kirshner, Aparna Raval, and Julie Lovchik for helpful discussions during the course of these studies. We also thank Drs. Fatah Kashchani, Shelby Berger, and Alfred Singer for critical review of the manuscript. We thank Josh Meyer for technical assistance.

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J. Biol. Chem. 2000, 275:10160-10167.
doi: 10.1074/jbc.275.14.10160

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