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Functional significance of the TATA element major groove in transcription initiation by RNA polymerase II

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ABSTRACT

The binding of TFIID to the TATA element initiates assembly of a preinitiation complex and thus represents one of the most important steps for transcriptional regulation. The fact that the TATA binding protein (TBP), a subunit of TFIID, exclusively contacts the minor groove of the TATA element led us to ask whether the major groove of the TATA element plays any role in transcription initiation or its regulation. Our results show that modifications of the major groove of the TATA element in the adenovirus major late promoter have no effect on TFIID binding affinity or on transcription in a cell-free system reconstituted with purified factors. However, major groove modifications do decrease the levels of both basal and activator-mediated transcription in unfractionated nuclear extracts, indicating that the intact structure of the major groove of the TATA element is functionally important for transcription initiation in a more physiological context.

INTRODUCTION

Transcription of mRNA-encoding genes involves assembly of RNA polymerase II and general initiation factors into a functional preinitiation complex (PIC). This process is initiated by binding of TFIID to the TATA element, which can be facilitated by TFIIA, and is followed either by stepwise interactions of TFIIB, RNA polymerase II/TFIIF, TFIIE and TFIIH or by concerted interactions of these components in a holoenzyme complex (reviewed in 1–5). Although the TATA binding polypeptide (TBP) subunit of TFIID is sufficient for a basal level of PIC assembly and function, both TBP-associated factors (TAFs) within TFIID and other cofactors are also necessary for the function of transcriptional activators in metazoans (reviewed in 2,3,5–7). In some cases activator functions have been correlated with effects on TFIID binding (8–13). Direct activator interactions with TFIID (reviewed in 6,7) and TFIIA (14,15) or with co-activators (e.g. PC4) that interact with these components (16) have been implicated in these effects.

While TFIID binding can be stimulated by interacting activators and co-activators, including TFIIA, the binding and function of TFIID (and the derived TBP) can also be inhibited by negative cofactors (17). These include cofactors (NC1 and NC2/DR1·DRAP1) that bind competitively with TFIIA to promoter-bound TBP, thus preventing productive TFIIB interactions (17–20), and an ATP-dependent cofactor (ADI/Mot1) that prevents or reverses the binding of TBP to the promoter (21). These factors have the capacity to repress both basal and activated transcription. Hence, they represent another point of control through repression and anti-repression mechanisms involving factors (e.g. TBP and interacting TAFs, TFIIA and TFIIB) bound at or near the TATA element.

Because of the significant role in transcription initiation and regulation played by factors interacting at the TATA element, there has been a strong emphasis on structural studies of the corresponding promoter complexes. Earlier hydroxyl radical and methylation interference analyses of TBP–TATA element complexes indicated that TBP interacts primarily with the minor groove of the TATA element (22,23). These results were confirmed by X-ray crystallographic studies which showed further that the TBP interactions both widen the minor groove and kink the DNA at each end of the TATA element, with a resulting bend in the overall path of the promoter DNA (24,25). As revealed by the structure of the TBP–TFIIB–TATA complex, this severe DNA distortion facilitates TFIIB binding through interactions with TBP and with the phosphodiester backbone both upstream and downstream of the TATA element (26). The structure of the TBP–TFIIA–TATA complex also shows non-overlapping interactions of TFIIA with TBP and with DNA sequences just 5′ of the TATA element (27,28).

Although the TBP component of TFIID is the primary promoter binding subunit and interacts mainly via the minor groove of the TATA element, this does not exclude the possiblity that TAFs, or any other general initiation factors or cofactors, might interact with the major groove of the TATA element to regulate formation or function of PICs. In order to investigate this question, we have employed templates with specific modification of the major groove of the TATA element to study effects on TBP/TFIID binding and on transcription in both purified and crude systems.

MATERIALS AND METHODS

Preparation of template

Plasmid 5GpML1 was generated from plasmid pML1 (29) after ligation of DNA fragments containing five Gal4 binding sites into *Pst*I and *Xba*I sites. A primer (designated primer a in Fig. 1)

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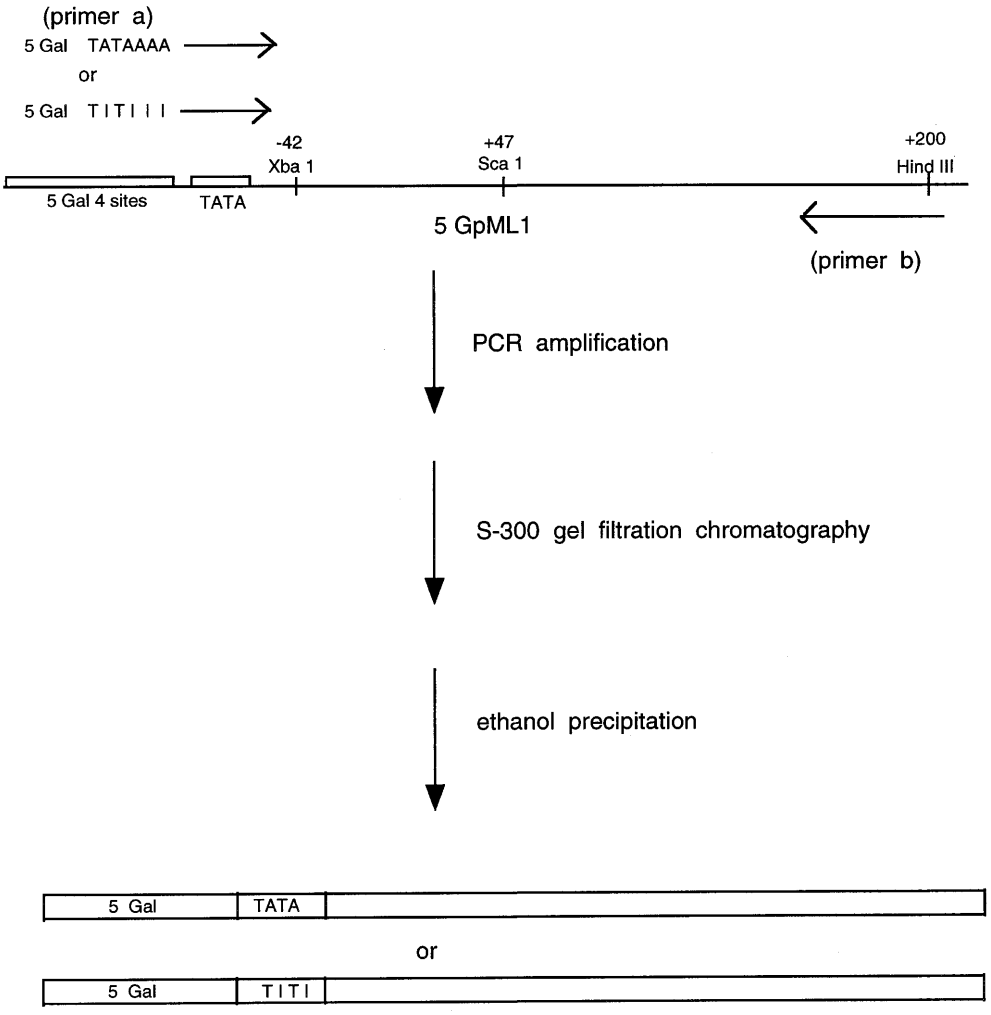

Figure 1. Schematic diagram of preparation of the wild-type and the IC base pair-substituted templates.

containing five Gal4 binding sites and the adenovirus major late promoter was prepared by two steps. DNA fragments containing five Gal4 binding sites were generated by PCR amplification from 5GpML1 and then cut (on one end) by *Xba*I. Oligodeoxynucleotides containing the wild-type sequences or IC base pair substitutions in the TATA element were synthesized and annealed to produce an *Xba*I overhang at one end. These two DNA fragments were ligated and the desired DNA strands were separated by denaturing polyacrylamide gel electrophoresis. Templates for transcription assays (Fig. 1) were prepared using the primer containing five Gal4 binding sites and the TATA element together with another primer. Primers and unincoorporated deoxyribonucleotides were removed by S-300 gel filtration chromatography and agarose gel electrophoresis and then the DNA was purified using a gel extraction kit from Qiagen. Sequences of PCR products were confirmed by Maxam–Gilbert sequencing.

Nuclear extracts and fractions

HeLa nuclear extract was prepared as described previously (30). To prepare TBP-depleted nuclear extract 500 µl nuclear extract was incubated for 2 h at 4°C with the same volume of agarose beads containing immobilized preimmune or anti-TBP immune antibodies. The salt concentration of the nuclear extract was adjusted to 0.4 M KCl to avoid co-immunoprecipitation of proteins that interact weakly with TBP. Agarose beads containing preimmune or immune antibodies were prepared by incubation of 500 µl swollen protein A–agarose and 500 ml preimmune or immune serum for 12 h. After washing with PBS, beads were incubated with bovine serum albumin (1 mg/ml) to block non-specific binding sites. They were then washed twice with 1 ml PBS and BC buffer (20 mM Tris–HCl, pH 7.8, at 4°C, 20% glycerol, 0.2 mM EDTA, 0.2 mM PMSF) containing 400 mM KCl before addition of nuclear extract.

General transcription factors and the USA cofactor fraction were prepared from HeLa nuclear extract as described (31). Fractions containing TFIIE/F/H were prepared by sequential chromatography on P11 (0.5 M KCl eluate), DE52 (0.3 M KCl eluate), double-stranded DNA–cellulose (flow-through) and FPLC Mono S (0.3 M KCl eluate) columns. Fractions containing USA (16) were prepared by sequential chromatography on P11 (0.85 M KCl eluate), DE52 (flow-through) and heparin–Sepharose (0.5 M KCl eluate) columns. TFIIA was purified on an Ni–resin affinity column as described

(32). Recombinant hexahistidine-tagged TFIIB was a generous gift from S.Malik (33). FLAG epitope-tagged human TBP and TFIID (34) were kindly provided by C.-M.Chiang.

Transcription assay

Transcription reactions with HeLa nuclear extract or with purified factors (16,35) were carried out as described. RNA was extracted with phenol/chloroform, incubated at 37°C for 2 h with a ^{32}P-labeled primer spanning +72 to +92 of the major late promoter (100 fmol, 1×10^7 c.p.m./pmol) and precipitated with ethanol. Primer extension reactions were carried out as described elsewhere (35). The cDNA products were extracted with phenol/chloroform, precipitated with ethanol and analyzed on a 7% polyacrylamide gel containing 8 M urea. Specific bands were quantitated by scintillation counting or by phosphorimager.

Electrophoretic mobility shift and footprinting assays

Radioactively labeled DNA fragments for footprinting assays were prepared by end-labeling of templates followed by digestion with *Hin*dIII (see Fig. 1). Footprinting analyses were performed as described previously (34). To prepare probes for DNA bending analyses the two ends (*Nsi*I for the 5′-end and *Apa*I for the 3′-end) of the synthetic oligo 55mers of the wild-type and the IC-substituted TATA elements were ligated into multicloning site fragments (100 bp) from pGEM-7Zf using *Nsi*I and *Apa*I restriction enzyme sites. Equivalent length (155 bp) DNA probes containing the TATA element in different locations of the DNA fragments were generated by digesting with different restriction enzymes (*Apa*I, *Xba*I, *Sma*I, *Bam*HI and *Nsi*I). DNA–protein complexes were separated on a 4% (40:1) polyacrylamide gel.

RESULTS

In order to selectively modify the major groove surface of the TATA element, a PCR procedure was used to prepare templates which contained inosine·cytosine (IC) base pairs in place of adenine·thymidine (AT) base pairs only in the TATA element (Fig. 1 and see Materials and Methods for details). Substitution of IC base pairs for AT base pairs has been reported to alter positions of hydrogen bond donor/acceptor groups of bases in the major groove but to leave those in the minor groove intact (36). The substitution of IC base pairs for AT base pairs at positions –30 and –28 in the adenovirus major late promoter TATA element (**T**A**T**AAAA) was technically impossible using this method. Therefore, substitutions at positions –29 and –27 to –24 (T**A**T**AAAA**) were carried out.

Effects of IC base pair substitutions in the TATA element on the DNA binding affinities of TBP and TFIID

Starr *et al.* (23) showed that substitution of IC base pairs for AT base pairs in the TATA element of the adenovirus major late promoter did not change the DNA binding specificity of TBP, although they did not determine DNA binding affinity of TBP with the IC base pair-substituted TATA element. A DNase I footprinting analysis was carried out in order to compare DNA binding affinities of TBP and TFIID with the wild-type versus the IC base pair-substituted DNAs. Results of the TBP footprinting assays with wild-type and IC base pair-substituted DNAs are shown in Figure 2a. Sensitivity of the TATA sequences to DNase I was enhanced in the IC base pair-substituted DNA, indicating possible changes in DNA structure at the TATA sequences. Nonetheless, both templates showed dose-dependent increases in protection around the TATA element that were similar and differed by <2-fold. Thus, as expected from crystallographic studies, modification of the TATA element major groove does not significantly change the apparent DNA binding affinity of TBP. No marked difference in the DNA binding affinities of TBP to the TATA or TITI elements was observed by EMSA (data not shown). In addition, the presence of TFIIA increased the affinities of TBP for the two templates to a similar extent (data not shown).

DNase I footprinting analyses of affinity-purified TFIID (34) bound to the adenovirus major late promoter (Fig. 2b) showed the typical pattern of protected regions and the hypersensitive sites reported before (37). As observed in the analysis with TBP, the sensitivity to DNase I around the TATA region was higher for the IC base pair-substituted probe than for the wild-type probe. Cleavage in the protected regions decreased to an equivalent degree on both templates as the amount of fTFIID was increased, again suggesting that substitution of IC base pairs for AT base pairs in the TATA element does not change the apparent DNA binding affinity of TFIID.

Although DNase I footprinting analyses showed that the IC base pair substitutions did not change the DNA binding affinity of TBP, the physical properties of the corresponding TBP–DNA complexes might differ. Crystallographic studies of the TBP–TATA element complex showed sharp kinks in the DNA resulting from insertion of two phenylalanine residues at the first T:A base pair and at the base step between the last 2 bp of the TATA element (24,25). On the premise that substitution of IC base pairs for AT base pairs in the TATA element might change the nature of hydrophobic interactions between phenylalanine residues and bases, DNA bending was analyzed to determine whether modification of the major groove in the TATA element might alter the bending center or the angles of the TBP–DNA complexes. To facilitate these studies, equal length DNA probes containing the TATA element in different locations of DNA fragments were generated using different restriction enzymes (see Materials and Methods for details). Changes in the bending angle or center can be detected by changes in the electrophoretic mobility of the TBP–DNA complexes on a native polyacrylamide gel (38). As shown in Figure 2c, the mobilities of the TFIIA–TBP–DNA complexes with wild-type and IC base pair-substituted probes did not appear to be markedly different. These results suggest that substitution of IC base pairs for AT base pairs in the TATA element induces little, if any, change in the bending angle or center of the TFIIA–TBP–DNA complexes. An analysis of DNA bending in TBP–DNA complexes lacking TFIIA also failed to show any difference between the two templates (data not shown).

Effect of the IC base pair substitutions in the TATA element on transcription in a reconstituted system

A crytallographic study of the TBP–TFIIB–DNA ternary complex also showed that TFIIB binding to the TBP–TATA element complexes did not alter TBP binding to the minor groove of the TATA element (26). The apparently equivalent ability of the IC base pair-substituted DNA and the wild-type DNA to form TBP–TFIIB–DNA ternary complexes (data not shown) led us to analyze transcription efficiency of the two templates using a reconstituted transcription system containing recombinant TFIIB,

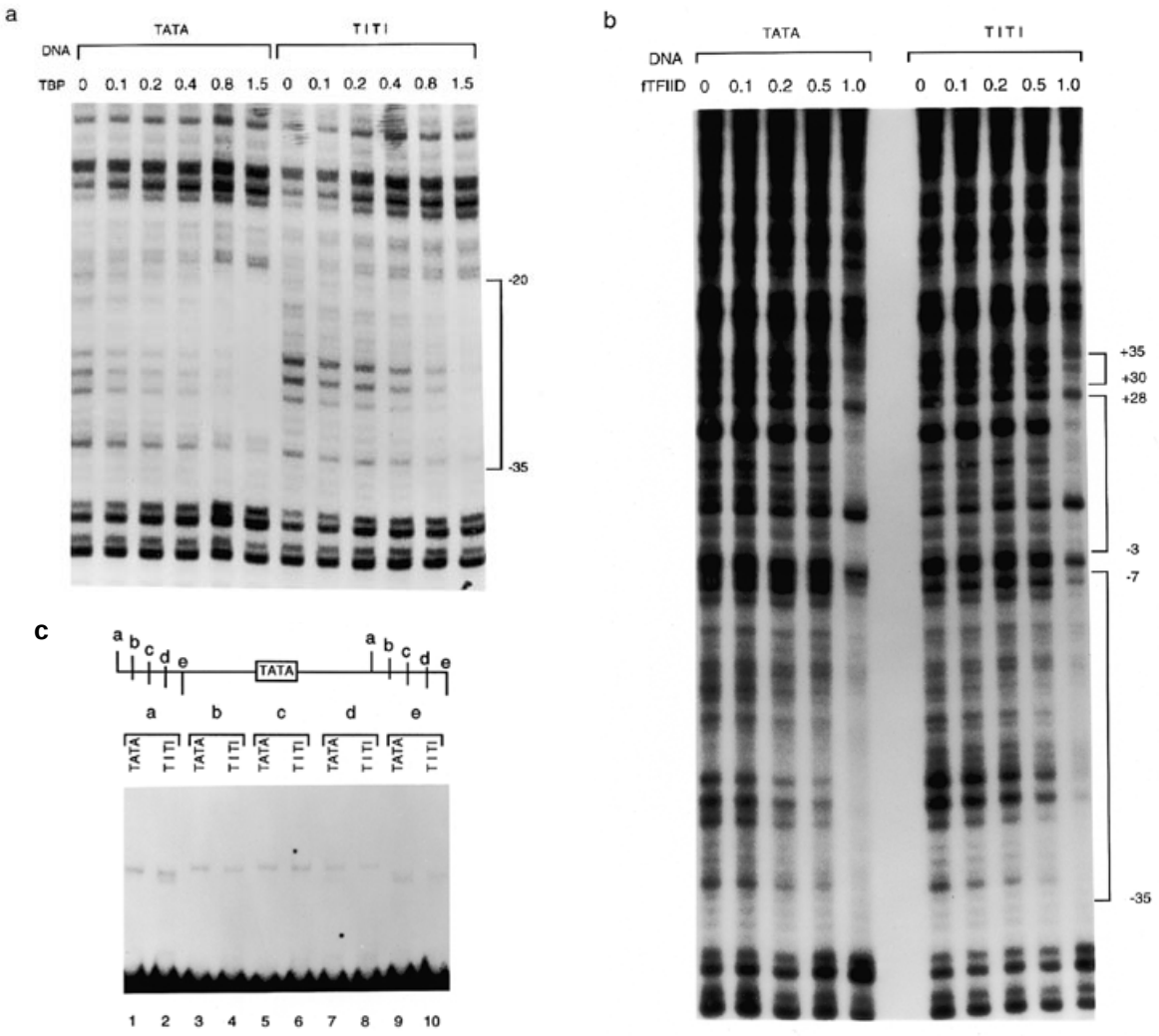

Figure 2. Substitution of IC base pairs for AT base pairs in the TATA element does not change DNA binding affinities of TBP or TFIID. PCR products were phosphorylated with [γ-^{32}P]ATP and then digested with *Hin*dIII. The 5′-end of non-transcribed strands was labeled. Probes were incubated with fTBP (**a**) or fTFIID (**b**) and digested with 0.2 ng DNase I for 2 min. The areas of protection are bracketed. For EMSA with permutated DNA fragments (**c**), 5 fmol probe were incubated with 10 fmol TBP and Ni–resin affinity-purified TFIIA (32) for 30 min at 30°C.

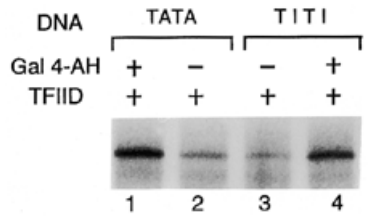

Figure 3. Basal and activated transcription in a reconstituted system with purified factors. Reactions contained wild-type (TATA) or IC base pair-substituted (TITI) linear templates, TFIIA, recombinant TFIIB, recombinant TBP or purified fTFIID (34), TFIIE/F/H, RNA polymerase II, USA and Gal4–AH as indicated.

affinity-purified TFIID and partially purified fractions containing TFIIA, TFIIE/F/H and USA (see Materials and Methods for details; 16). In the absence of any activator TFIID-dependent basal transcription from the two templates showed a <2-fold difference (Fig. 3, lanes 2 and 3). Thus, modification of the major groove in the TATA element did not change the efficiency of basal level transcription in this system, consistent with the observation of efficient formation of TBP–TFIIB–DNA ternary complexes on IC base pair-substituted templates (data not shown). When the activator Gal4–AH was added to the reconstituted system, the overall transcription directed by TFIID was enhanced to a comparable extent (~3- to 4-fold) on both templates (lanes 1 and 4). The results of a primer extension transcription assay (Fig. 3) also showed that the IC base pair-substituted templates direct transcription initiation from the same site as the wild-type template. Therefore, IC base pair substitutions in the TATA element allow for both competent PIC formation and efficient transcription initiation with general transcription factors and activators.

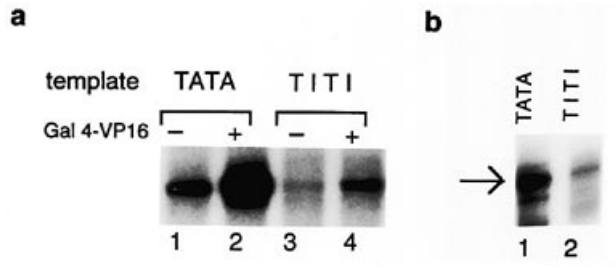

Figure 4. Transcription assay in HeLa nuclear extract (**a**) and yeast nuclear extract (**b**). Each transcription reaction (25 ml) contained 50 µg nuclear extract, 150 fmol wild-type (TATA) or IC base pair-substituted (TITI) linear template and 200 ng Gal4–VP16 as indicated.

Effect of IC base pair substitutions in the TATA element on transcription in nuclear extracts

In order to analyze the transcription efficiency of the IC base pair-substituted templates in the presence of a more natural complement of cellular repressors and/or co-activators, transcription assays were carried out in unfractionated HeLa nuclear extract. In contrast to the results obtained with the more purified reconstituted system, IC base pair substitutions in the TATA element decreased the efficiency of basal level transcription 6- to 8-fold in HeLa nuclear extract (Fig. 4a, lane 1 versus lane 3). As with the purified reconstituted system, the primer extension analysis also showed that the IC base pair-substituted template directs transcription initiation at the same site as the wild-type template. Thus, in the crude system substitution of IC base pairs for AT base pairs in the TATA element changes the efficiency, but not the accuracy, of transcription initiation. The same differences in transcription efficiency were observed with increases or decreases in the concentrations of the templates (data not shown). IC base pair substitutions in the TATA element of the adenovirus E1b promoter also resulted in a decreased level of basal transcription (data not shown). Since the E1b promoter is much weaker than the major late promoter and contains a different TATA element (TATATAAT), the decreased efficiency of transcription resulting from major groove modifications appears to be an intrinsic property of the TATA element that is not strictly dependent either upon promoter strength or specific TATA sequences.

To examine whether transcriptional activators could compensate for the decreased transcription efficiency of the IC base pair-substituted templates, promoter-specific activators were added to the nuclear extract. Consistent with the results observed in the purified reconstituted system, the acidic activator Gal4–VP16 enhanced transcription (Fig. 4a, compare lanes 1 and 2 with 3 and 4). However, the overall degree of enhancement (~4-fold) was comparable for the two templates, such that the absolute level of activation for the IC base pair-substituted templates was less than observed with the wild-type template. Similar results were observed in transcription assays with Gal4–SP1, containing the glutamine-rich activation domain of SP1 (data not shown). Altogether, these results show that while modifications of the major groove in the TATA element do not decrease the efficiency of core promoter transcription with the relatively pure reconstituted system, the intact structure of the major groove of the TATA element is fuctionally important both for optimal core promoter function (basal transcription) and for optimal activator-enhanced transcription in a HeLa nuclear extract.

In order to analyze whether decreased transcription efficiency resulting from IC base pair-substituted TATA elements was species specific, transcription assays were also performed with a yeast nuclear extract. The results in Figure 4b show that transcription of the IC base pair-substituted template was also decreased by 7-fold in the yeast nuclear extract. Thus, the decreased transcription efficiency by modification of the major groove is not species specific. Therefore, modifications of the major groove in the TATA element result in a failure to counteract what appears to be an evolutionarily conserved inhibitory factor(s).

Effect of TFIID versus TBP on transcription of the IC base pair-substituted templates in nuclear extract

Since transcription in nuclear extracts is presumably driven by TFIID rather than by TBP, it was necessary to determine whether the decreased level of transcription was dependent upon the TAF components of TFIID. Therefore, TFIID was immunodepleted from the HeLa nuclear extract using an anti-TBP antibody and the transcriptional efficiencies of wild-type and IC base pair-substituted templates in response to exogeneously added TFIID or TBP were determined. Immunodepletion of TFIID/TBP was >95% as determined by Western blotting with antisera against human TBP (data not shown) and the immune-depleted extract showed no transcription with either wild-type or IC base pair-substituted templates (Fig. 5, lanes 1 and 2). In contrast, the results observed with preimmune-depleted nuclear extract (Fig. 5, lanes 7 and 8) are consistent with the decreased level of transcription of the IC base pair-substituted templates shown in Figure 2. Addition of affinity-purified FLAG epitope-tagged TFIID to the TFIID/TBP-depleted nuclear extract restored transcription, although the wild-type template was transcribed ~7-fold more efficiently than the IC base pair-substituted template (Fig. 5, lanes 3 and 4). Addition of TBP to the TFIID/TBP-depleted nuclear extract also restored transcription, again with a comparable (5-fold) difference between the wild-type and IC base pair-substituted templates (lanes 5 and 6). Thus, substitution of IC base pairs for AT base pairs in the TATA element decreased transcriptional efficicency with both TBP and TFIID in the nuclear extract, suggesting that decreased transcription efficiency of the IC base pair-substituted templates is not TAF dependent.

The results thus far show that modification of the major groove of the TATA element supports efficient formation of the PIC and, therefore, supports efficient transcription initiation with the relatively purified factors. However, the efficiency of basal as well as activated transcription was decreased in the nuclear extract. One possible explanation for the discrepancy in transcription efficiency of the IC base pair-substituted template in a relatively purified reconstituted system versus a crude nuclear extract might be that certain inhibitory proteins (potentially non-specific) in the nuclear extract more efficiently destabilize or interfere with PIC assembly or function on the IC base pair-substituted template. It is conceivable that such an inhibitory protein(s) could have a higher affinity for the modified major groove of the IC base pair-substituted templates, in the context of the PIC, and thus decrease the overall efficiency of transcription initiation. An alternative and more interesting possibility is that the major groove modifications reduce interactions of other

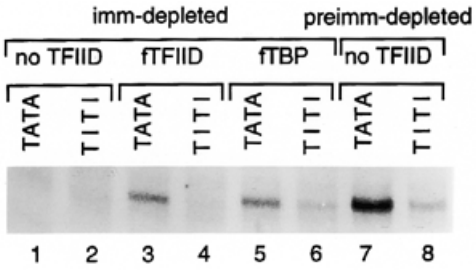

Figure 5. IC base pair-substituted templates decrease the efficiency of transcription directed either by TFIID or TBP. TFIID/TBP was depleted from the preimmune- or immune-depleted nuclear extract with anti-TBP antibodies as described in Materials and Methods. Transcription reactions contained equimolar amounts of TBP (10 ng) or highly purified epitope-tagged TFIID and wild-type (TATA) or IC base pair-substituted templates.

factors that in turn counteract the activity of more specific negative cofactors in the nuclear extract. The former hypothesis was tested by incubation of nuclear extract with a synthetic double-stranded oligonucleotide containing the IC base pair substitutions and a resulting TITI element, which would be expected to titrate the presumptive inhibitory protein(s). In order to prevent the binding of non-specific inhibitory proteins to the synthetic TITI-containing oligonucleotide, the nuclear extract was first preincubated with poly(dG-dC). As expected from the previous demonstration that TBP/TFIID interacts with the TITI element, an increase in the amount of oligonucleotide decreased the transcriptional signal on both templates (Fig. 6). If the nuclear extract contains inhibitory proteins that have a higher affinity for the modified major groove one might expect that transcription from the IC base pair-substituted template would differ from that of the wild-type template upon addition of the oligonucleotide. However, as shown in Figure 6, addition of the oligonucleotide decreased transcription from both templates to the same degree. This suggests that, if present, the putative inhibitory protein may not interact directly with the modified major groove itself. However, the experiment does not eliminate the possibility that the presumptive inhibitory protein(s) might recognize the modified major groove within the context of a DNA–transcription factor complex.

The human negative cofactor NC2 (or DR1·DRAP1) was reported to interact stably with TBP/TFIID–DNA complexes and, consequently, to prevent formation of a productive PIC and to decrease both basal and activated levels of transcription (17–20). Since the IC base pair-substituted template decreases basal as well as activated transcription, we analyzed whether NC2 shows a higher affinity for TBP complexes on DNA containing IC base pair substitutions in the TATA element. NC2 was depleted from the nuclear extract using anti-NC2β (DR1) antisera. Western blotting and transcription assays showed that this treatment efficiently removed TFIID as well as NC2, indicating that NC2 is associated with TFIID in unfractionated nuclear extracts (data not shown). Thus, it was necessary to add ectopic TBP to the depleted nuclear extract. As shown in Figure 7, depletion of NC2 did not result in a preferential increase in transcription from the IC base pair-substituted template. Hence, NC2 appears not to be the putative inhibitory protein(s) which decreases transcription efficiency from the IC base pair-substituted template.

DISCUSSION

The fact that TBP interacts exclusively in the minor groove of the TATA element leaves open the possibility that the cognate major groove might be a potential target for other factors in regulating the functions of TFIID or interacting factors at or near the TATA element. An important distinction between the major and minor grooves in the TATA element is that positions of functional groups at the minor groove surface do not discriminate AT→TA transversions, whereas those in the major groove do (36). Therefore, it is possible that different TATA sequences provide the same functional surfaces in the minor groove, for TBP interaction, but different functional surfaces in the major groove.

In order to analyze the hypothesis that the major groove of the TATA element is functionally important in regulating TBP/TFIID binding or function by other factors, substitution of IC base pairs for AT base pairs in the TATA element was employed to selectively modify the major groove surface of the TATA element. Modification of the TATA element major groove by IC base pair substitution shows little or no change in the apparent DNA binding affinities of TBP or TFIID. This result excludes the possibility that the TAF components of TFIID might interact with the major groove of the TATA element or alter TBP binding to the minor groove of the TATA element. In addition, the IC base pair-substituted TITI element was able to form TBP–TFIIA–TFIIB complexes as efficiently as the wild-type TATA element (data not shown), consistent with the results of a crystallographic study showing that TFIIB binding to a TBP–TATA element complex does not alter TBP binding in the minor groove of the TATA element (26). In further studies with the remaining general transcription initiation factors (TFIIE/F/H) and RNA polymerase II, it was found that the TITI element could support efficient formation of a complete PIC (data not shown). Thus, modifications of the major groove in the TATA element did not seem to interfere with formation of a PIC comprised

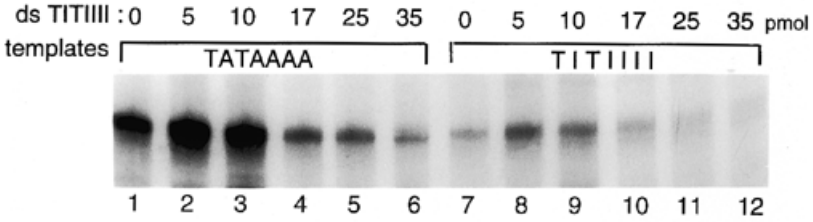

Figure 6. Preincubation of nuclear extract with a double-stranded oligonucleotide containing the TITI element had no effect on repression by major groove modifications. HeLa nuclear extract was incubated with wild-type (TATA) or IC base pair-substituted (TITI) oligonucleotide (positions –50 to –15 of the major late promoter) at 30°C for 20 min, before addition of wild-type or IC base pair-substituted templates and NTPs and a further 1 h incubation.

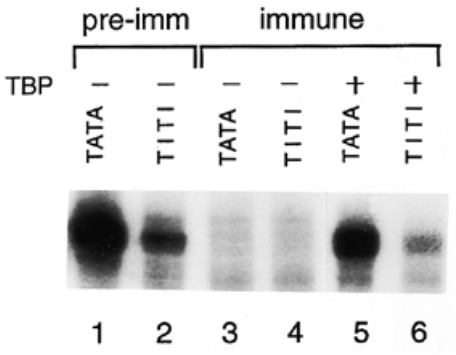

Figure 7. Removal of NC2 from nuclear extracts does not prevent impaired transcription activity with the IC base pair-substituted templates. NC2- and TFIID-depleted nuclear extract was prepared as described in Materials and Methods. Transcription reactions contained TBP (10 ng) and wild-type (TATA) or IC base pair-substituted templates.

of well-studied general initiation factors. As expected from results showing efficient formation of the PIC on the template containing the TITI element, the modifications showed little effect (<2-fold) on TFIID-dependent transcription, either basal or activator dependent, in the purified reconstituted system.

In contrast to the results observed in the reconstituted system, the major groove modifications markedly decreased (6- to 8-fold) the efficiency of basal as well as activated transcription in the nuclear extract. Addition of highly purified initiation factors such as TFIIA, TFIID, TFIIE/F/H and recombinant TFIIB did not enhance the decreased transcription efficiency with the IC base pair-substituted template (data not shown), suggesting that a decreased transcription efficiency was not due to a limited amount of initiation factors. A decreased transcription efficiency that is specific to nuclear extracts relative to purified reconstituted systems has at least two obvious explanations. First, there may be certain inhibitory proteins specific to nuclear extracts that bind more efficiently to the modified major groove, in the context of the PIC, to interfere with binding or function of TFIID or interacting factors. Second, major groove interactions may be required for certain positive cofactors to modify or reverse the activity of other more physiological negative cofactors, analogous to NC2 or Mot1, that may be enriched in nuclear extracts relative to purified systems. Although the present data argue against an involvement of NC2, the possible involvement of the less well-characterized NC1 (39) or the human homolog (M.Timmers, personal communication) of yeast ADI/Mot1 (21) was not eliminated. Further, recent studies in yeast indicate still other negative cofactors (43–45) that could have human homologs.

At this stage the identity of the factor(s) responsible for decreased transcription of the IC base pair-substituted TITI element in nuclear extracts remains unclear. However, it is clear that the intact structure of the major groove of the TATA element is more important for efficient transcription initiation by RNA polymerase II in nuclear extracts, presumably a more physiological context, than in purified reconstituted systems. Similarly, phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II is selectively required in crude systems, relative to a more purified reconstituted system, for transcription of the adenovirus major late promoter (46) and the yeast ADC1, CYC1, GAL1 and HIS5 promoters (47). Recent studies have shown that cofactors in the RNA polymerase II holoenzyme complex interact with the CTD, such that CTD phosphorylation might reverse these interactions (reviewed in 4). Should some of these factors have inhibitory effects on the general transcription factors, this could explain the lack of a requirement for CTD phosphorylation in the purified system lacking these cofactors. The current studies suggest a parallel situation for negative cofactors or their antagonists acting via major groove interactions at the TATA element.

Given existing information on the structure of the PIC (reviewed in 6), it is relevant to ask about the potential for factor interactions in the major groove of the TATA element in the context of the PIC. Although no basal factor contacts have been detected by the various (crystallographic, photocrosslinking and mutational) studies, consistent with the results of the present study with purified factors, the TBP-mediated DNA bend and the TBP-interacting factors (TFIIA and TFIIB) could restrict access of other factors to the major groove (26–28,40–42). Nonetheless, major groove interactions of other factors (or even basal factors) may still be possible, especially during the early stages of PIC assembly (e.g. before binding of both TFIIA and TFIIB) or if the structure of the PIC is dynamic. In any case, our results showing decreased transcription efficiency by modification of the major groove in the TATA element provide an additional insight into another possible step in the regulation of transcription.

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