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Role of TATA box sequence and orientation in determining RNA polymerase II/III transcription specificity

Yan Wang, Richard C. Jensen and William E. Stumph*

Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182-1030, USA

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ABSTRACT

Work from a number of laboratories has indicated that the TATA box sequence can act as a basal promoter element not only for RNA polymerase II (RNAP II) transcription, but also for transcription by RNA polymerase III (RNAP III). We previously reported that, in the absence of other *cis*-acting elements, the canonical TATA sequence TATAAAAA specifically supported transcription by RNAP II in an unfractionated *Drosophila* nuclear extract, whereas the sequence TTTTTATA (the same sequence in reverse orientation) directed RNAP III transcription. We have now examined a variety of other TATA box sequences with regard to RNA polymerase selectivity and their ability to support RNAP III transcription. The results have allowed us to rank these TATA box sequences with respect to their relative strengths as RNAP III promoter elements in unfractionated *Drosophila* extracts. Further, the data indicate that T residues at positions 2 and 4 of the TATA box appear to be important determinants of RNAP III selectivity in this system, whereas A residues at these positions favor RNAP II transcription. Finally, the data suggest that transcription factors TFIID and TFIIIB, although both capable of binding a variety of TATA elements, have distinct sequence preferences for recognizing the TATA box and possibly the surrounding DNA.

INTRODUCTION

The eukaryotic RNA polymerase (RNAP) II and RNAP III transcriptional machineries are evolutionarily related. Indeed, some of the polypeptides utilized by the two RNA polymerases are identical. For example, the TATA box-binding protein (TBP) is required for both RNAP II and RNAP III transcription as a subunit of the polymerase-specific factors TFIID and TFIIIB respectively [reviewed in (1,2)]. Moreover, at least five polypeptide subunits are shared in common by the RNAP II and RNAP III enzymes of yeast (3,4). A number of homologous yet non-identical polypeptides are also characteristic of the two systems. Examples include the RNAP II-specific factor TFIIB and the homologous RNAP III-specific factor BRF (5–8), as well as a number of evolutionarily related integral subunits of the RNA polymerases themselves (3).

Although the TATA box was originally identified as a promoter element upstream of many mRNA genes, it is also capable of directing transcription by RNAP III (9–16). Interestingly, work from our lab recently demonstrated that, in the absence of other *cis*-acting elements, the RNA polymerase specificity of a promoter could be determined by the orientation of a certain TATA box sequence (17). In an unfractionated *Drosophila* nuclear extract, the canonical TATA sequence TATAAAAA specifically promoted RNAP II transcription, whereas the same sequence when flipped into the reverse orientation (TTTTTATA) specifically promoted RNAP III transcription. Moreover, in the same reaction mixture, RNAP II initiated transcription ~22–23 base pairs (bp) downstream of the forward TATA box, whereas RNAP III initiated a similar distance from the TATA box but in the opposite 'upstream' direction (17). Those results indicated that the RNAP II and RNAP III initiation complexes preferentially assembled in opposite orientations on the chosen TATA box sequence.

The sequence TATAAAAA was selected for the above-described studies based upon its high degree of sequence asymmetry and upon a previous observation that it acted as a strong RNAP II promoter in the *Drosophila in vitro* transcription system (18). We now report related studies using a number of different TATA box sequence variants. The data have enabled us to rank the TATA boxes in terms of their abilities to promote RNAP III transcription in the unfractionated *Drosophila* nuclear extract. The results of these studies indicate that the original sequence TTTTTATA may represent an optimal RNAP III TATA box and that Ts at positions 2 and 4 are important determinants of RNAP III specificity in this system.

MATERIALS AND METHODS

Plasmid templates

Plasmid templates H/D 'forward' TATA and H/D 'reverse' TATA have been previously described (17). Each contains the synthetic sequence TATAAAAA inserted between the *Kpn*I and *Bam*HI sites of pUC18, but oriented in opposite directions. All the other

* To whom correspondence should be addressed

H/D templates were constructed by replacing the TATA sequence between the *Kpn*I and *Bam*HI sites of the H/D 'forward' TATA plasmid with other synthetic TATA variants. This generated a family of plasmids that were identical to each other except for the 8 bp TATA box sequence.

The wild-type *Drosophila* U1 gene template with 391 bp of 5′-flanking DNA has been previously described (18). The wild-type U6 gene template was prepared starting from a plasmid obtained from Ram Reddy (Baylor College of Medicine) that contained the wild-type *Drosophila* U6-2 gene (19). An *Eco*RI–*Hae*III restriction fragment was isolated that contained 59 bp of U6-2 gene coding sequence and 409 bp of 5′-flanking DNA. This fragment was then inserted between the *Eco*RI and *Hinc*II sites of pUC18 to use as a template for *in vitro* transcription assays. All plasmids were grown in *E.coli* TOP10 cells (Invitrogen), purified using Qiagen Plasmid Maxi kits, and sequenced to confirm the identity of the promoter region.

In vitro transcription

Transcription reactions, purification of transcription products, and analysis by primer extension were as described previously (17). The ^{32}P-labeled primers were complementary to pUC18 DNA on either side of the polylinker (New England Biolabs no. 1233 or a primer [1211z] similar to New England Biolabs 1211, but extended by 7 nucleotides at its 5′-end). Primer extension products were separated in 10% denaturing polyacrylamide gels that were dried prior to autoradiography and/or PhosphorImager quantitation. Each template was transcribed in a minimum of five different experiments.

RESULTS

Studies on 'canonical' TATA boxes: effect of sequence and orientation on promoter strength and RNA polymerase specificity

In an initial experiment, several canonical variants of the TATA sequence and their reverse-orientation counterparts were tested for their ability to promote transcription by either RNAP II and/or RNAP III. These synthetic 8 bp TATA sequences were cloned within the pUC18 vector polylinker region (see top of Fig. 1). Toward the right (or 'downstream') of the TATA element each template contained synthetic sequence that corresponds to a combination of sequences surrounding the *Drosophila* U1 and U6 gene transcription start sites. Toward the left of the TATA box (in the 'upstream' direction), the templates contained sequence resembling a combination of the human U1 and U6 gene transcription start sites. Knowing that U1 genes are transcribed by RNAP II and U6 genes by RNAP III, we chose this combination of U1 and U6 sequences with the goal of optimizing the context for initiation of transcription for each of the RNA polymerases. A second important consideration in choosing these sequences was the knowledge that both the U1 and U6 genes have external promoters that reside entirely upstream of position –20; thus there are no known promoter elements within the synthetic U1/U6-like sequences that were employed to optimize the transcription start sites.

The DNA templates were transcribed *in vitro* using a soluble nuclear fraction (SNF) prepared from *Drosophila* embryos (20,21). The drug α-amanitin was used as a specific inhibitor of RNAP II. Because insect RNAP III is resistant to inhibition by even a high concentration of α-amanitin (22,23), tagetitoxin was employed as a specific RNAP III inhibitor (24). Transcription products were assayed by primer extension analysis.

Figure 1, lanes 1–32, shows typical results using an oligonucleotide primer (1211z) that detects transcription initiating in the '*Drosophila*' sequences and proceeding in the rightward direction. The sequence TATAAAAA and the cognate reverse TATA sequence, TTTTTATA, produced results identical to previously reported findings (17). Both TATA sequences supported transcription in the *Drosophila* SNF with similar efficiencies (Fig. 1, compare lane 1 with 18, and lane 19 with 32). However, the canonical forward sequence TATAAAAA promoted RNAP II-specific transcription (inhibited by α-amanitin but not by tagetitoxin, lanes 2 and 3) whereas the cognate reverse TATA sequence TTTTTATA specifically promoted transcription by RNAP III (not inhibited by α-amanitin but inhibited by tagetitoxin, lanes 20 and 21). Transcription was TATA box-dependent, since no products were detectable when a sequence with three G/C substitutions was inserted in either orientation (lanes 17 and 31).

As expected, two other canonical forward TATA sequences, TATATAAA and TATAAATA, overwhelmingly supported RNAP II transcription (Fig. 1, lanes 5–12). However, when these two TATA sequences were placed in the reverse orientation, a mixture of both RNAP II and RNAP III transcription was observed, and the overall level of transcription was somewhat decreased (Fig. 1, lanes 23–30). Transcription promoted by the sequence TTTATATA was more thoroughly inhibited by tagetitoxin than by α-amanitin, indicating that 60–70% of the transcription was due to RNAP III (lanes 23–26). The second sequence, TATTTATA, promoted RNAP II and RNAP III transcription with approximately equal efficiencies (lanes 27–30). Somewhat surprisingly, the completely symmetrical TATA box, TATATATA, very efficiently promoted transcription by RNAP II but worked poorly for RNAP III transcription (Fig. 1, lanes 13–16). Indeed, by PhosphorImager analysis, the RNAP II signal was >15-fold greater than the RNAP III signal. Together, these results suggest that increasing the number of alternating TpA residues disfavors transcription by RNAP III relative to RNAP II.

Next, transcription initiating in the upstream direction (in the synthetic 'human' DNA) was measured for each of these constructs by using the 1233 oligonucleotide for the primer extension assays (Fig. 1, lanes 33–64). Since transcription was assayed in the opposite direction, the TATA boxes can be considered to be reversed in orientation relative to the experiments shown in lanes 1–32. In accord with previous results (17), transcription initiating in the reverse direction from TATAAAAA was predominantly due to RNAP III (lanes 33–36). However, the data shown in lanes 37–44 was somewhat unexpected because transcription promoted by these TATA boxes in the leftward direction was almost entirely due to RNAP II. These results should be compared with those shown in lanes 23–30, in which TATA boxes with the same sequences (relative to the direction of transcription) promoted a *mixture* of RNAP II and RNAP III transcription. Thus, there appears to be an intrinsic bias in favor of RNAP II over RNAP III transcription in the leftward direction relative to transcription in the rightward direction, but the underlying cause of this bias has not been investigated. Apparently a strong RNAP III TATA box (TTTTTATA) can overcome this bias (lanes 33–36), whereas TATA boxes that are less selective are unable to do so (lanes 37–44). The symmetrical TATA sequence TATATATA acted as a very strong RNAP II-specific promoter in

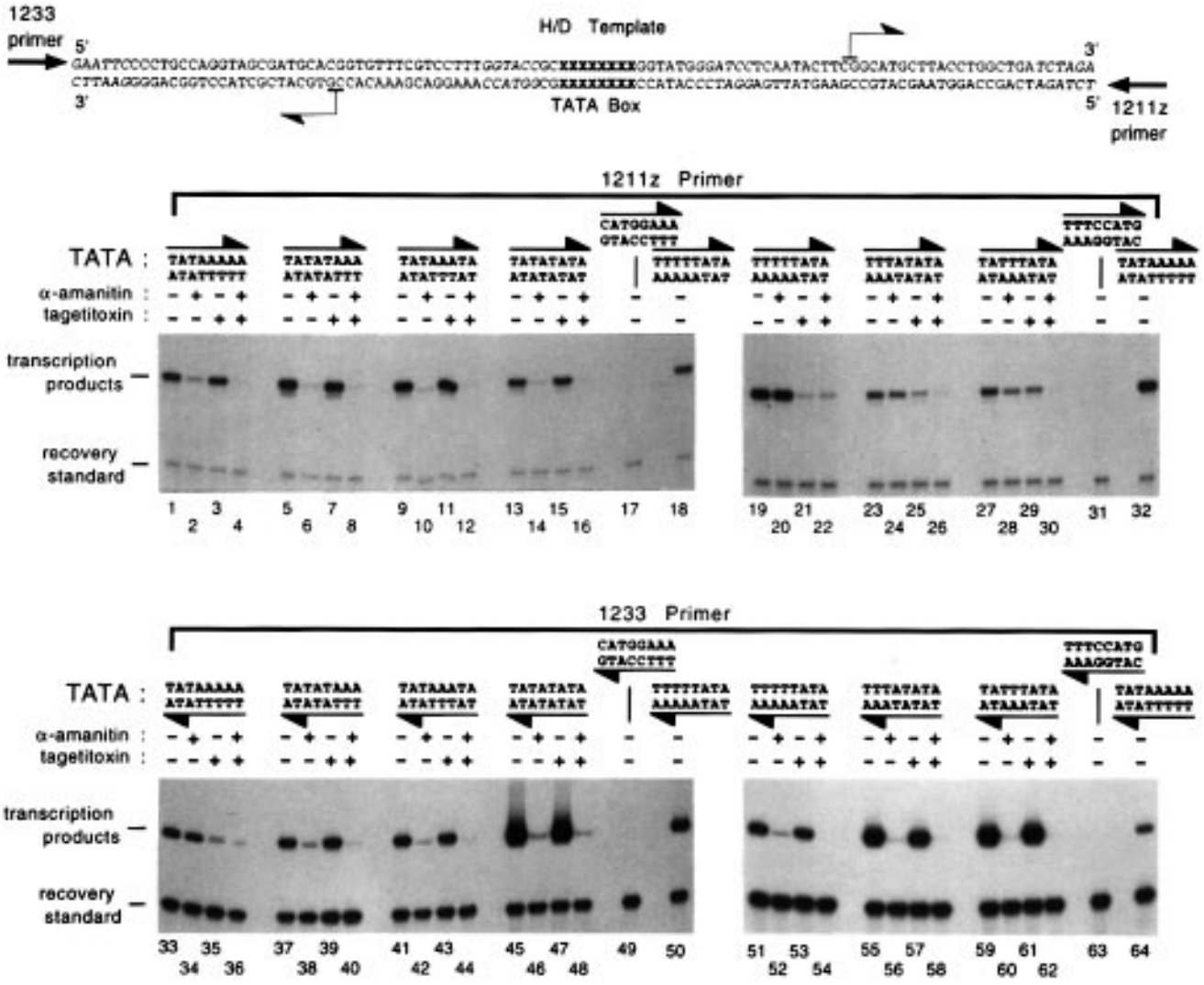


Figure 1. Primer extension analysis of *in vitro* transcription products synthesized from H/D templates that contain various TATA box sequences in 'forward' and 'reverse' orientations. Transcription was carried out using an unfractionated *Drosophila* nuclear extract. Templates were constructed containing the DNA sequences shown at the top of the figure inserted between the *Eco*RI and *Xba*I sites of pUC18. Italics represent plasmid-derived DNA. The bold Xs indicate the position where the variant TATA box sequences were positioned. The actual TATA sequences utilized in each construction are shown above the corresponding lanes of the autoradiograms. The arrows above or below the TATA box sequences represent the direction of transcription being assayed. Plus signs above the individual lanes indicate inclusion of the RNAP II-specific inhibitor α-amanitin (2 μg/ml) or the RNAP III-specific inhibitor tagetitoxin (0.4 U/ml, Epicentre Technologies). Transcription start sites (indicated by arrows above or below the sequence at the top of the figure) were mapped by running the primer extension products alongside sequencing ladders (data not shown). Bands on the autoradiograms are indicated that correspond to reverse transcription products of 76–77 (1211z primer) or 71–72 nucleotides (1233 primer). The position of a 54mer oligonucleotide that was added to each reaction as a recovery standard is also shown. Each panel represents a different set of transcriptions, but reactions with the original forward TATA (TATAAAAA) and reverse TATA (TTTTTATA) templates were included in each panel to permit a comparison of intensities from set to set.

the leftward direction (lanes 45–48 in comparison to lanes 13–16), lending further support to the concept that there is a bias favoring RNAP II transcription in the leftward direction. As expected, the TATA boxes in the canonical forward orientation (relative to the leftward direction of transcription being assayed) were highly specific for RNAP II (lanes 51–62).

Effect of an A to G mutation at position 2 of the TATA box

The substitution of a G residue for the canonical A residue at position 2 of the TATA box has been shown to be highly deleterious for TATA-mediated RNAP II transcription (25) and more recently for TATA-mediated RNAP III transcription (11,26). Constructs containing this mutation (TGTAAAAA) were therefore analyzed for their ability to support transcription by either RNAP II or RNAP III (Fig. 2). A comparison of lanes 1 and 2 (Fig. 2) shows that the G substitution abolished nearly all transcription (due to RNAP II) in the forward (rightward) direction. Interestingly, the mutant TATA box, when reversed in orientation, was able to support RNAP III-specific transcription in the rightward direction (lanes 3–6), although at an ~5-fold reduced level compared to the non-mutant reverse TATA box (lane 7). When transcription was assayed in the leftward direction (lanes 8–14), similar results were obtained. Whereas RNAP II

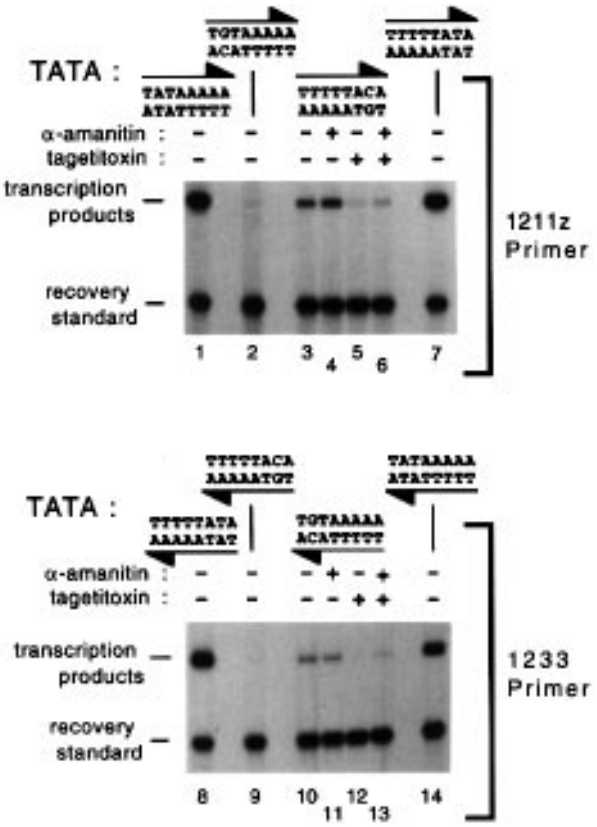


Figure 2. Primer extension analysis of *in vitro* transcription products from H/D 'forward' and 'reverse' TGTAAAAA templates. Conditions and explanation of symbols are the same as described in the legend to Figure 1.

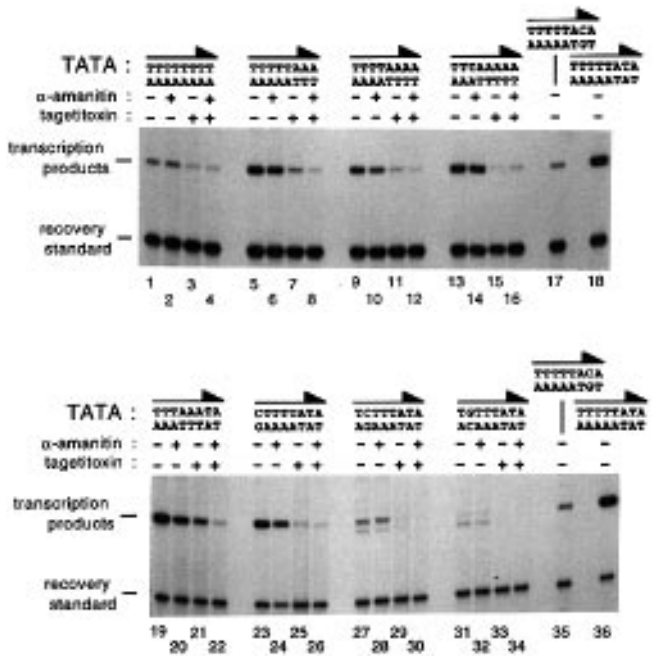


Figure 3. Primer extension analysis of *in vitro* transcription products synthesized from H/D templates containing a variety of TATA sequences. All reactions in this figure utilized the 1211z oligonucleotide for primer extension. Other conditions and explanation of symbols are the same as described in the legend to Figure 1.

transcription was reduced over 30-fold by the base substitution (compare lanes 8 and 9), RNAP III transcription promoted by the mutated reverse TATA box was reduced only 5-fold relative to the original reverse TATA sequence (lanes 10–14).

Determinants of RNAP III specificity

One interpretation of the above experiments is that a run of five Ts on the non-template strand in the upstream portion of the TATA box (e.g. TTTTTATA or TTTTTACA) represents a sufficient determinant for RNAP III transcription. Therefore, in the next series of experiments (Fig. 3), we tested the ability of a number of variant TATA sequences to promote transcription by RNAP III. To avoid any variation that might arise from the contribution of the flanking sequences, these experiments were done only with the 1211z primer to assay transcription only in the rightward direction.

A run of eight consecutive T residues (Fig. 3, lanes 1–4) supported RNAP III transcription but with only ~10% of the efficiency of the sequence TTTTTATA (compare with lane 18). The sequences TTTTTAAA, TTTTAAAA and TTTAAAAA also exhibited a high degree of selectivity in favor of RNAP III versus RNAP II transcription (Fig. 3, lanes 5–16). The sequence TTTAAATA preferentially supported RNAP III transcription, but in this case a significant RNAP II component (~30% of the total transcription signal) was clearly detectable (Fig. 3, lanes 19–22).

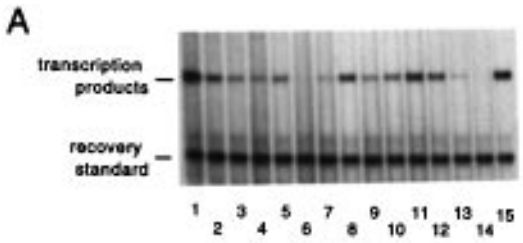
The sequence CTTTTATA, which is the reverse orientation of the adenovirus major late canonical TATA box, was a reasonably strong and highly selective promoter for RNAP III (Fig. 3, lanes 23–26). Two variants with changes at the second position (TCTTTATA and TGTTTATA) supported very low levels of transcription, although the low level that remained was RNAP III-specific. (In this particular experiment, a shorter product apparently resulting from downstream initiation was detected from these two templates, but this shorter transcript was not consistently observed in other experiments.)

Relative strength of various TATA box sequences as promoters for RNAP III transcription

In order to rank the various TATA box variants according to their ability to promote RNAP III transcription *in vitro*, 14 of the constructs were transcribed in the presence of α-amanitin and the products were run together in the same gel (Fig. 4A). Band intensities from five experiments similar to that shown in Figure 4A were quantified by PhosphorImager analysis, and the levels of transcription were determined relative to that obtained with the sequence TTTTTATA. In Figure 4B, the TATA sequences are ordered according to their relative strengths as promoters for RNAP III transcription. From these data, it is evident that the sequence TTTTTATA represents the optimal RNAP III TATA sequence among those tested. Moreover, there is a wide range of RNAP III transcription efficiencies that is dependent upon the specific TATA box sequence. These results will be further addressed in the Discussion section.

The synthetic templates are transcribed *in vitro* with efficiencies comparable to native U1 and U6 promoters

A final experiment was performed to assess the physiological relevance of the *in vitro* transcriptional levels obtained from the



B

Lane	TATA	Relative Pol III Efficiency		
1,15	TTTTTATA	1.00		
11	TTTAAATA	.38	+/-	.04
8	TTTTTAAA	.36	+/-	.04
12	CTTTTATA	.34	+/-	.02
2	TTTATATA	.29	+/-	.03
10	TTTAAAAA	.28	+/-	.02
3	TATTTATA	.22	+/-	.02
5	TTTTTACA	.18	+/-	.03
9	TTTTAAAA	.16	+/-	.02
4	TATATATA	.12	+/-	.01
7	TTTTTTTT	.10	+/-	.01
13	TCTTTATA	.10	+/-	.01
6	TTTCCATG	.05	+/-	.01
14	TGTTTATA	.01	+/-	.01

Figure 4. (**A**) Autoradiogram showing the relative efficiencies of RNAP III transcription promoted by various TATA box sequences. All transcription reactions were performed in the presence of 2 µg/ml α-amanitin. The TATA sequences driving transcription in each lane are indicated in the first and second columns of part (B). (**B**) Results from several experiments similar to the one shown in (A) were quantified by PhosphorImager analysis. The TATA sequences are arranged in descending order of RNAP III transcription efficiency relative to the sequence TTTTTATA. Errors shown are the standard deviation of the mean.

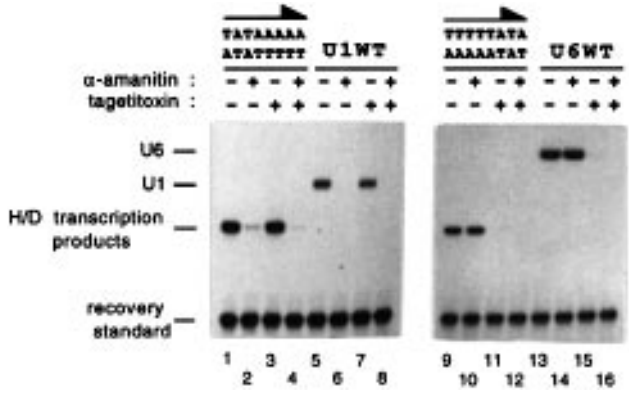


Figure 5. Comparison of the *in vitro* transcription activities of synthetic H/D templates with the activities of natural U1 and U6 gene promoters. All analyses were carried out using the 1211z oligonucleotide for primer extension. Products arising from transcription of the wild-type U1, wild-type U6 and H/D templates are indicated. Other conditions and explanation of symbols are described in the legend to Figure 1.

artificial templates relative to the transcriptional activities of natural promoters recognized by RNAP II and RNAP III. Figure 5 compares the activity of the H/D 'forward' TATA template (lanes 1–4) with the activity of the native *Drosophila* U1 gene promoter (lanes 5–8). The synthetic template was transcribed ~3-fold more efficiently *in vitro* by RNAP II than the template that contained the wild-type U1 gene promoter.

Figure 5 also presents data for RNAP III-specific transcription. The relative transcription levels obtained from the H/D 'reverse' TATA template (lanes 9–12) were compared with levels obtained from the wild-type U6 gene promoter (lanes 13–16). The U6 promoter was transcribed ~2-fold more efficiently than the reverse TATA promoter. In conclusion, transcription from the synthetic templates *in vitro* occurred at levels that were reasonably comparable to those obtained from the natural U1 and U6 gene templates.

DISCUSSION

The TATA box as a promoter element for RNAP III transcription

We have examined the ability of various TATA box sequences to selectively promote RNAP II or RNAP III transcription in a *Drosophila* nuclear extract. Our data indicate that canonical forward TATA box sequences (e.g. TATAAAAA, TATATAAA or TATAAATA) are strongly selective for RNAP II transcription. The cognate reverse TATA box, in one case, was highly selective for RNAP III (TTTTTATA), and in the other two cases (TTTATATA and TATTTATA) RNAP II and/or RNAP III transcription was preferentially supported depending upon context (i.e. polymerase specificity was dependent upon direction of transcription assayed). On the other hand, the symmetrical sequence TATATATA strongly favored RNAP II transcription in both directions of transcription. These results argue that T residues at positions 2 and 4 in the TATA box act as important determinants for RNAP III specificity in the crude *Drosophila* nuclear extract.

Several TATA box variants were found to be preferentially selective for RNAP III. However, none of these was as efficient for promoting RNAP III transcription as the original sequence first studied in earlier experiments (TTTTTATA) (17). Although the library of mutations tested is certainly not exhaustive, several conclusions can be drawn from the available data and can be summarized as follows.

Position 1. Substitution of C for T (CTTTTATA) decreased RNAP III-specific transcription ~3-fold (Fig. 4), yet the selectivity for RNAP III versus RNAP II remained very high (Fig. 3).

Position 2. Data for all four nucleotides were obtained at the second position and the results indicate the following order of nucleotide preference for effective RNAP III transcription: T > A > C > G (compare TTTTTATA, TATTTATA, TCTTTATA and TGTTTATA in Fig. 4).

Position 3. No comparative data is available since all TATA variants tested contained T at this position.

Positions 4 and 5. A TpT dinucleotide appears to be optimal; RNAP III transcription from templates with either an ApT or TpA dinucleotide was significantly less efficient. Interestingly an ApA double substitution partly restored a higher level of expression (compare TTTTTATA versus TTTATATA versus TTTAAATA, and compare TTTTTAAA versus TTTTAAAA versus TTTAAAAA in Fig. 4).

Positions 6 and 8. The functional role of the nucleotides at these positions is addressed by a double point substitution (TTTTTTTT versus TTTTTATA). In this case, RNAP III transcription was reduced 10-fold, indicating that transcriptional efficiency by RNAP III can be drastically affected by the specific nucleotides at positions 6 and/or 8 of the TATA box.

Position 7. The importance of this position is revealed by the fact that an A or C substitution resulted respectively in either a 3- or 6-fold decrease in RNAP III transcription (TTTTTATA versus TTTTTAAA versus TTTTTACA in Fig. 4). This result is consistent with the recent observation that a seventh-position C substitution (in the TATA box context TATAAACA or TATTTACA) was extremely detrimental to TATA-mediated RNAP III transcription in a reconstituted yeast system (26). In the *Drosophila* SNF, the effect appears to be not quite as severe; alternatively, perhaps the five Ts in the upstream portion of our sequence (TTTTTACA) produce a stronger RNAP III TATA box and partially overcome the detrimental effect of the C at position 7.

The contextual (flanking sequence) contribution to RNA polymerase specificity was not examined in this study. The templates were designed to minimize known RNAP II or RNAP III promoter elements other than the TATA box, but it is possible that cryptic elements may affect RNA polymerase specificity in a particular direction. This is evidenced by the fact that the sequences TTTATATA and TATTTATA promoted a mixture of RNAP II and III transcription in the rightward direction in our experiments, but when inverted favored nearly exclusive RNAP II transcription in the leftward direction (Fig. 1). This is not unreasonable in that the RNAP II and RNAP III basal transcription machineries contain polypeptide subunits in addition to TBP that directly contact the DNA in the pre-initiation complex, and in some instances these interactions exhibit sequence-specificity. For example, *Drosophila* TFIID interacts with both a consensus initiation site sequence and with a downstream promoter element (DPE) present in many TATA-less *Drosophila* mRNA-encoding genes (27). The RNAP II-specific factor TFIIB contacts DNA on each side of the TATA box (28,29); by analogy, the evolutionarily homologous RNAP III-specific factor BRF may make similar contacts with the DNA. The B″ subunit of TFIIIB contains a putative DNA-binding domain (30), and X-ray crystallography has revealed that TFIIA directly contacts DNA (31,32). Thus, multiple subunits of the basal transcription machinery may contribute to specific DNA recognition. In spite of this, transcription of our constructs by both RNAP II and RNAP III was highly TATA box-dependent since certain TATA mutations (e.g. TGTAAAAA, CATGGAAA and TTTCCATG) reduced transcription to essentially non-detectable levels for both RNA polymerases. Moreover, the quantitative comparisons of various TATA sequences as promoters for RNAP III transcription (Figs 3 and 4) were performed by assaying transcription in only the rightward direction. Since the TATA variants were present in identical environments in these constructions, the differences in the activities of these templates reflect the net effects of the specific TATA sequences that they contain.

RNAP III versus RNAP II specificity

In this report we have examined the ability of a number of TATA box variants to preferentially support either RNAP II or RNAP III transcription in an unfractionated soluble nuclear extract that contains both polymerase activities. In general, our results suggest that runs of T residues in the first five positions of the TATA box favor RNAP III transcription, whereas alternating T–A residues favor RNAP II transcription. In an earlier paper, we interpreted these data to indicate that a 'forward' TATA box favored RNAP II transcription, and a 'reverse' TATA box RNAP III transcription (17). One mechanism to explain this phenomenon is that TBP binds in opposite orientations on RNAP II and RNAP III promoters (15,17). Alternatively, different TBP associated factors (TAFs) in TFIID and TFIIIB may differentially modulate the DNA binding specificity of TBP or themselves contribute to TATA box recognition. In this case, the sequence of the TATA box, rather than the orientation *per se*, may be the feature responsible for polymerase selection. Indeed, when complexed with TAFs in the RNAP I-specific factor SL1, TBP is unable to recognize a TATA box (33). Additional examples in which the DNA binding specificity of TBP may be modified by associated factors have been noted by other workers (26,34,35). Moreover, Whitehall *et al.* (26), using a yeast recombinant RNAP III system, have recently presented evidence that TBP binds in the same orientation on the yeast U6 RNAP III promoter as it does on RNAP II promoters.

The data presented here are compatible with either model of TBP binding. Whether the preferences for RNAP II or RNAP III transcription observed in our experiments arise from TBP interacting in opposite orientations or alternatively arise from the differential action of TAFs in different TBP complexes can only be determined by further experimentation. A definitive answer will undoubtedly require high-resolution cross-linking experiments or the comparative topographical mapping of polypeptides in partially assembled RNAP II and RNAP III pre-initiation complexes. Significantly, however, our results provide an insight into the preferred functional DNA binding specificities of TFIIIB relative to that of TFIID.

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REFERENCES

1 Sharp,P.A. (1992) *Cell*, **68**, 819–821.
2 Hernandez,N. (1993) *Genes Dev.*, **7**, 1291–1308.
3 Sentenac,A., Riva,M., Thuriaux,P., Buhler,J.-M., Treich,I., Carles,C., Werner,M., Ruet,A., Huet,J., Mann,C., Chiannilkulchai,N., Stettler,S. and Mariotte,S. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, Plainview, NY, pp. 27–54.
4 McKune,K. and Woychik,N.A. (1994) *Mol. Cell. Biol.*, **14**, 4155–4159.
5 Buratowski,S. and Zhou,H. (1992) *Cell*, **71**, 221–230.
6 Colbert,T. and Hahn,S. (1992) *Genes Dev.*, **6**, 1940–1949.
7 Lopez-De-Leon,A., Librizzi,M., Puglia,K. and Willis,I.M. (1992) *Cell*, **71**, 211–220.
8 Wang,Z. and Roeder,R.G. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 7026–7030.
9 Joazeiro,C.A.P., Kassavetis,G.A. and Geiduschek,E.P. (1994) *Mol. Cell. Biol.*, **14**, 2798–2808.
10 Huet,J., Conesa,C., Manaud,N., Chaussivert,N. and Sentenac,A. (1994) *Nucleic Acids Res.*, **22**, 2282–2288.
11 Gerlach,V.L., Whitehall,S.K., Geiduschek,E.P. and Brow,D.A. (1995) *Mol. Cell. Biol.*, **15**, 1455–1466.

12 Chung,J., Sussman,D.J., Zeller,R. and Leder,P. (1987) *Cell*, **51**, 1001–1008.
13 Bentley,D.L., Brown,W.L. and Groudine,M. (1989) *Genes Dev.*, **3**, 1179–1189.
14 Mitchell,M.T., Hobson,G.M. and Benfield,P.A. (1992) *J. Biol. Chem.*, **267**, 1995–2005.
15 Mitchell,M.T. and Benfield,P.A. (1993) *J. Biol. Chem.*, **268**, 1141–1150.
16 Huang,W.B., Wong,J.M. and Bateman,E. (1996) *Nucleic Acids Res.*, **24**, 1158–1163.
17 Wang,Y. and Stumph,W.E. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 8606–8610.
18 Zamrod,Z., Tyree,C.M., Song,Y. and Stumph,W.E. (1993) *Mol. Cell. Biol.*, **13**, 5918–5927.
19 Das,G., Henning,D. and Reddy,R. (1987) *J. Biol. Chem.*, **262**, 1187–1193.
20 Kamakaka,R.T., Tyree,C.M. and Kadonaga,J.T. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 1024–1028.
21 Kamakaka,R.T. and Kadonaga,J.T. (1994) *Methods Cell Biol.*, **44**, 225–235.
22 Sklar,V.E.F., Jaehning,J.A., Gage,L.P. and Roeder,R.G. (1976) *J. Biol. Chem.*, **251**, 3794–3800.
23 Lewin,B. (1994) *Genes V.* Oxford University Press, New York.
24 Steinberg,T.H., Mathews,D.E., Durbin,R.D. and Burgess,R.R. (1990) *J. Biol. Chem.*, **265**, 499–505.
25 Wobbe,C.R. and Struhl,K. (1990) *Mol. Cell. Biol.*, **10**, 3859–3867.
26 Whitehall,S.K., Kassavetis,G.A. and Geiduschek,E.P. (1995) *Genes Dev.*, **9**, 2974–2985.
27 Burke,T.W. and Kadonaga,J.T. (1996) *Genes Dev.*, **10**, 711–724.
28 Lee,S. and Hahn,S. (1995) *Nature*, **376**, 609–612.
29 Nikolov,D.B., Chen,H., Halay,E.D., Usheva,A.A., Hisatake,K., Lee,D.K., Roeder,R.G. and Burley,S.K. (1995) *Nature*, **377**, 119–128.
30 Aasland,R., Stewart,A.F. and Gibson,T. (1996) *Trends Biochem. Sci.*, **21**, 87–88.
31 Tan,S., Hunziker,Y., Sargent,D.F. and Richmond,T.J. (1996) *Nature*, **381**, 127–134.
32 Geiger,J.H., Hahn,S., Lee,S. and Sigler,P.B. (1996) *Science*, **272**, 830–836.
33 Beckmann,H., Chen,J.L., O'Brien,T. and Tjian,R. (1995) *Science*, **270**, 1506–1509.
34 Lee,D.K., DeJong,J., Hashimoto,S., Horikoshi,M. and Roeder,R.G. (1992) *Mol. Cell. Biol.*, **12**, 5189–5196.
35 Heard,D.J., Kiss,T. and Filipowicz,W. (1993) *EMBO J.*, **12**, 3519–3528.