MOLECULAR AND CELLULAR BIOLOGY, July 1997, p. 3809–3816 Vol. 17, No. 7
0270-7306/97/$04.00+0

Transcription Reinitiation Rate: a Special Role for the TATA Box

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Received 31 October 1996/Returned for modification 19 December 1996/Accepted 8 April 1997

Promoters need to specify both the timing of transcriptional induction and the amount of transcript synthesized. In order to explore each of these effects separately, in vitro assays for the level of active preinitiation complex formation and for the rate of continuous RNA production were done. The effects were found to be influenced differently by different promoter elements. A consensus TATA element had a very strong effect on the rate of continuous RNA production, whereas two types of activators were important primarily in forming active transcription preinitiation complexes. Consensus TATA promoters exhibited high rates of continuous transcription; they assembled active preinitiation transcription complexes slowly but then produced transcripts continuously at an approximately fivefold-higher rate. Initiator-containing TATA-less promoters produced continuous transcripts slowly. Point mutations in the TATA element led to lower levels of transcription by reducing the number of preinitiation complexes and amplifying this reduction by lowering the apparent reinitiation rate. The results allow understanding of the sequence diversity of promoter elements in terms of specifying separate controls over the sensitivity of gene induction and over the strength of the induced promoter.

The DNA elements that comprise RNA polymerase II promoters should serve two functions. First, they need to specify under what circumstances transcription will be activated. Thus, they cause appropriate activation during development, physiological induction, or cell cycle progression. The primary means of activation is thought to be at the level of assembly of functional preinitiation complexes (for recent reviews, see references 11, 13 and 34). Second, the elements should specify the amount of transcript produced from the activated promoter. Once a gene is activated, the amount of its transcript is determined primarily by the number of transcription reinitiation events. Fully induced promoters can differ in the amount of RNA produced, indicating that promoter elements can influence promoter strength separately from how they influence induction. The potential separation of the roles of elements in stimulation of induction and in promoter strength has not been explored in depth.

It has been suggested that the controls over RNA polymerase II initiation and reinitiation can be uncoupled (14), raising the possibility that the two processes can have different requirements. Related issues have been discussed for all three types of eukaryotic RNA polymerases (7, 14, 24). For initiation and reinitiation requirements to differ, the processes must use different pathways to produce transcripts, as is well known for RNA polymerase III (17). For RNA polymerase II, different pathways were first suggested by in vitro studies that showed that a transcribed template appeared to be preferentially used through multiple rounds, in contrast to templates that have not been transcribed previously (12). The source of this preference was thought to be that TFIID remained bound to the template after initial polymerase escape and promoter clearance, thus facilitating formation of the new assemblies used for transcription reinitiation (12, 35). Very recent studies confirm that TFIID can be left behind after initiation (27, 41).

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Such data indicate that reinitiation need not recapitulate every step used in initiation and allow for the possibility that reinitiation could be more rapid than initial transcription. In prior studies (14) of the activated adenovirus E4 promoter, reinitiation did occur faster than expected; it was several times faster than the formation of preinitiation complexes from free components. Rate studies using fractionated transcription factors at this promoter suggest that this high reinitiation rate may be a consequence of factors being left behind during each round of transcription. This is suggested by the observation that if E4 templates are assembled with GAL-AH, TFIID, and TFIIA, then the subsequent events leading to transcription occur very rapidly (37). On the other hand, studies using a *Drosophila* promoter and transcription factors found that initiation and reinitiation occurred at the same rate (16).

These observations indicate that the potential for facilitated reinitiation exists but suggest that it need not be universal. One likely source of diversity in reinitiation events could be the type of promoter elements that are present, and this is a major focus of the present work. RNA polymerase II promoters generally contain a series of regulatory elements along with some combination of TATA and initiator (inr) elements (30). All of these elements are thought to contribute to promoter strength, as defined generically by their contribution to the production of RNA in vitro and in vivo. The continuous rate of RNA production is defined primarily by transcription reinitiation (see reference 34), and the roles of the various elements in this rate have not been investigated thoroughly. In principle, different elements could play differing roles in induction of preinitiation complexes and in setting the strength of promoters through reinitiation rates. These issues remain largely unexplored and are important because they are at the heart of why each promoter has a unique sequence.

We address these issues by establishing a quantitative in vitro assay for reinitiation and applying it to promoters that differ in their elements. Previously, we compared activated and basal transcription in test promoters containing consensus

TATA box and inr elements with or without six Sp1 binding sites. The activator was found to work by increasing stability during preinitiation complex formation without significantly altering the rate at which such complexes form or initiate (40). We began this new work by comparing the known rate of assembly of preinitiation complexes with the rate of reinitiation and then systematically varying the types of elements present. The results show that at certain of these promoters, reinitiation can be much faster than initiation. The data show that the TATA box is much more important for this facilitated reinitiation than either activator or inr elements, in contrast to the effects of these elements on formation of preinitiation complexes. The results indicate that promoters may be built with separate controls for induction and promoter strength, with the nature of the TATA sequences playing a special role in specifying promoter strength through the reinitiation pathway.

MATERIALS AND METHODS

Templates. The parental templates contain, as indicated, combinations of six simian virus 40 GC boxes, a consensus TATA element, and the inr element of the terminal deoxynucleotidyl transferase (TdT) gene (plasmids V, VI, and VII in reference 32). In some experiments, the GC boxes were replaced with either one, two, or five copies of GAL4 binding sites (5), which were used in conjunction with the activator GAL-AH (14). A shorter version of GC-TATA-inr, used in the competition experiments described below, was made by deleting 23 bp downstream of the inr element (B. Wolner, University of California, Los Angeles). In addition, a series of templates with mutated TATA boxes replacing the consensus TATA in the context of the GC-TATA-inr promoter was constructed. To construct these templates, oligonucleotides with the desired mutations were synthesized, and these served as upstream primers in PCRs along with a common oligonucleotide hybridizing about 400 bp downstream. For example, mutant 1 used upstream primer CGGAATTCGGGCTTTAAAAGGGGGTGGGGG GAG with downstream primer GTGCCACCTGACGTCTAAG. The other TATA (underlined) sequences are indicated in the text. The PCR products were cut with enzymes *Aat*II and *Eco*RI and ligated back into the parental plasmid (GC-TATA-inr) from which the same region had been removed. The sequences for all constructs were confirmed. For study of the mouse dihydrofolate reductase (DHFR) promoter, plasmid pSS625 was used as specified elsewhere (10).

In vitro transcription. For all templates except pSS625 (see below), the reaction mixtures contained 20 μl of HeLa cell nuclear extract in appropriate solution (8), 8.25 mM Mg^{2+}, 20 ng of supercoiled whole plasmid template (unless otherwise indicated), and 500 ng of pGEM as carrier DNA in a final volume of 40 μl. Incubations were done at 30°C. In preliminary transcription assays, a 60-min preincubation was followed by 2- and 30-min pulses with 500 μM nucleoside triphosphates (NTPs); extracts that exhibited severalfold differences in the two signals, indicating that they supported extensive reinitiation, were chosen. For continuous reinitiation assays, RNA samples were removed and analyzed at the subsequent times indicated below. RNA products were detected by reverse transcriptase extension of labeled primer CCTTATGTATCATACACATACG ATTTAGG, which hybridizes to up to nucleotide position +79. All templates were constructed so that a common extension product was detected. For pSS625 (DHFR promoter), the reaction conditions were as described previously (10). Briefly, 200 μM ATP and 500 μM (each) GTP, CTP, and UTP were used. Reactions were done at 24°C. RNA generated from this promoter was detected by labeled primer CACGGCGACGATGCAGTTCAATGGTC. Hybrid GAL activator-driven transcription was detected as described previously (14). These labeled cDNA products were separated on a 6% polyacrylamide–urea gel and visualized and quantified with a PhosphorImager (Molecular Dynamics, Inc.). In all cases, the pixel counts are used to quantify RNA, with no attempt made to determine the absolute number of moles of transcript.

Challenge experiments. Preinitiation complexes were allowed to form on 20 ng of the test template during a 1-h incubation. This time was chosen as it known that longer incubations do not significantly increase the use of templates, implying that all available templates are in preinitiation complexes. Then 400 ng of the challenge template was added, together with 500 μM NTPs. Incubation was continued for the various times indicated below. RNA from the test DNA was quantified by primer extension as described above. The challenge template was a short version of GC-TATA-inr in all cases (constructed by B. Wolner). Transcripts generated by this challenger can be measured with the same primer, but they are 23 nucleotides shorter than that made by test DNA.

RESULTS

Separate evaluations of the rate of reinitiation and the level of complex formation. In a prior study, we measured the rate at which preinitiation complexes form and then go on to initiate transcription. We found that preinitiation complexes form with a half time of 20 to 25 min and then can fully initiate transcription within approximately 1 min after nucleotides are added (40). These rates were measured by using promoters containing consensus TATA and TdT inr elements, either with or without six Sp1 sites. The initial goal of this study was to measure how rapidly reinitiation occurs at these promoters. To do this, a protocol in which multiple rounds of transcription occur and may be measured is needed. In preliminary assays (not shown), we found that very small amounts of DNA are essential to this protocol, presumably so that there are sufficient factors to support multiple rounds of reinitiation. We found that titration of plasmid DNA in the range from 10 to 40 ng gave proportional increases in transcription (Fig. 1), whereas larger amounts of DNA led to smaller increases (not shown). This shows that functional initiation factors are in excess in reactions using 20 ng of plasmid. Thus, in order to assure that the reaction is fully template limited, the assay uses only 20 ng of supercoiled plasmid template per reaction mixture (equivalent to 0.25 fmol per μl), far less than the amount typically used, especially in the study of basal templates. In prior experiments involving reinitiation in a HeLa extract, a nearly 60-fold-greater molar amount of promoter was used, and fewer rounds of reinitiation occurred (12).

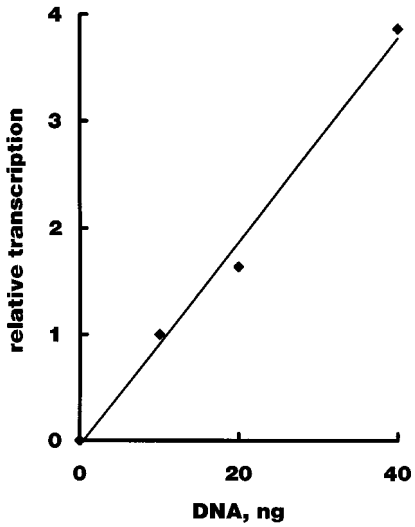

FIG. 1. The GC-TATA-inr supercoiled promoter plasmid was transcribed by using the indicated amounts, and the levels of RNA produced in 60-min free-transcription reactions are plotted. Similar experiments showed that 20 ng of the GC-inr promoter also gave template-limited reactions. Use of DNA amounts larger than 100 ng gave lesser increases.

The preinitiation complexes (initially using the GC-TATA-inr template) are formed by a 1-h incubation in the absence of nucleotides. It was shown previously that this time is sufficient for active preinitiation complex formation to reach its maximum capacity; further incubation can increase template usage by only approximately 5% (Fig. 2 in reference 40), as is known for other promoters (15, 39). The nucleotides are then added under conditions where a synchronous first round of transcription is known to be completed within 2 min; prior experiments show a saturation curve occurring over this time (Fig. 3 in reference 40). Thus, the protocol takes preformed active preinitiation complexes in the presence of excess initiation factors (Fig. 1) and induces them to synchronously produce transcript. The transcripts produced in this first round and at

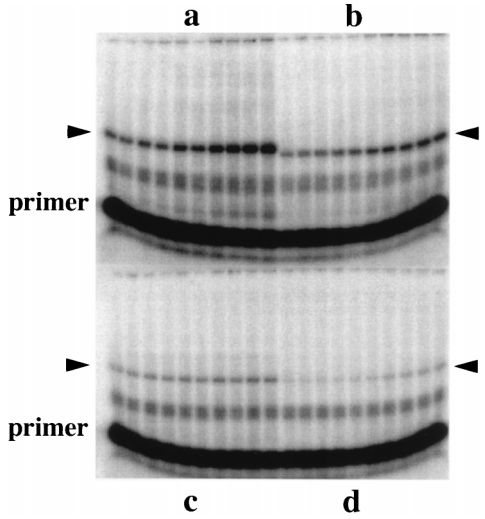

FIG. 2. In vitro transcription of four templates. After a 60-min preincubation of GC-TATA-inr (a), GC-TATA (b), GC-inr (c), or TATA-inr (d), transcription was initiated by supplying NTPs. For each panel, duplicate reactions were stopped at 2, 5, 10, 20, and 30 min (from left to right) and RNA was isolated. The 79-nucleotide-long radioactive cDNA products (arrowheads) were then separated on a 6% polyacrylamide–urea gel and analyzed with a PhosphorImager. The experiment was repeated two to four times with similar results.

subsequent times are monitored and quantified by PhosphorImager analysis of polyacrylamide gel separations.

The data (Fig. 2a and Fig. 3a, top curve) confirm the expected initial burst of RNA production, corresponding to the first round of transcription from the preformed active preinitiation complexes. Prior experiments have shown that such bursts are accompanied by total depletion of open transcription complexes (14). This burst is followed by a linear increase over a 30-min time course. Because the data of Fig. 1 show that free transcription factors are in excess, the continuous phase does not result from the use of limiting transcription factors suddenly freed during the burst phase. Instead, as the burst synthesis is template limited and involves depletion of open complexes, the postburst phase should correspond to the freeing of the template for reinitiation events. Thus, the linear increase is taken to correspond to the continuous transcription reinitiation events that can occur after the first RNA polymerases have left the promoter (similar to that seen in related protocols in references 12 and 39 except that the current study uses lower concentrations of DNA to ensure that the reactions are template limited).

The amount of RNA produced during these 30 min is at least five times the amount produced in the initial 2-min burst, indicating at least an additional five rounds of transcription. Estimation of the actual number of rounds requires an accurate measure of the amount of RNA produced in the first-round burst. We compared several methods for estimating this amount of RNA produced in the first round of transcription. This amount is approximately that measured at the earliest time point, taken just after the initial burst (2 min), and thus the 2-min level constitutes a simple estimate. In principle, some reinitiation may have occurred during these 2 min. If so, then one should extrapolate the curve back to the time of promoter clearance to obtain a slight correction. We showed previously that this time was about 1 min for the GC-TATA-inr promoter (40) and is in the range of 30 s to 2 min for the promoters analyzed below (Fig. 4a and data not shown). As an alternative measure of the amount of RNA produced in the first round, we ran separate experiments in which NTPs were added to preinitiation complexes for 1 or 2 min, either with or without the addition of 0.08% Sarkosyl to prevent reinitiation. We found that the use of triplicate reaction mixtures with a 2-min NTP chase gave the highest reliability, with the extrapolation and Sarkosyl methods yielding comparable results. We recommend this method to avoid potential complications due to modest but potentially variable complex disruption by Sarkosyl.

From the amount of RNA produced in the first-round burst, the results indicate that slightly more than five rounds of transcription followed the initial round of synthesis. It takes 30 min for these rounds of transcription to occur, and the quantitative

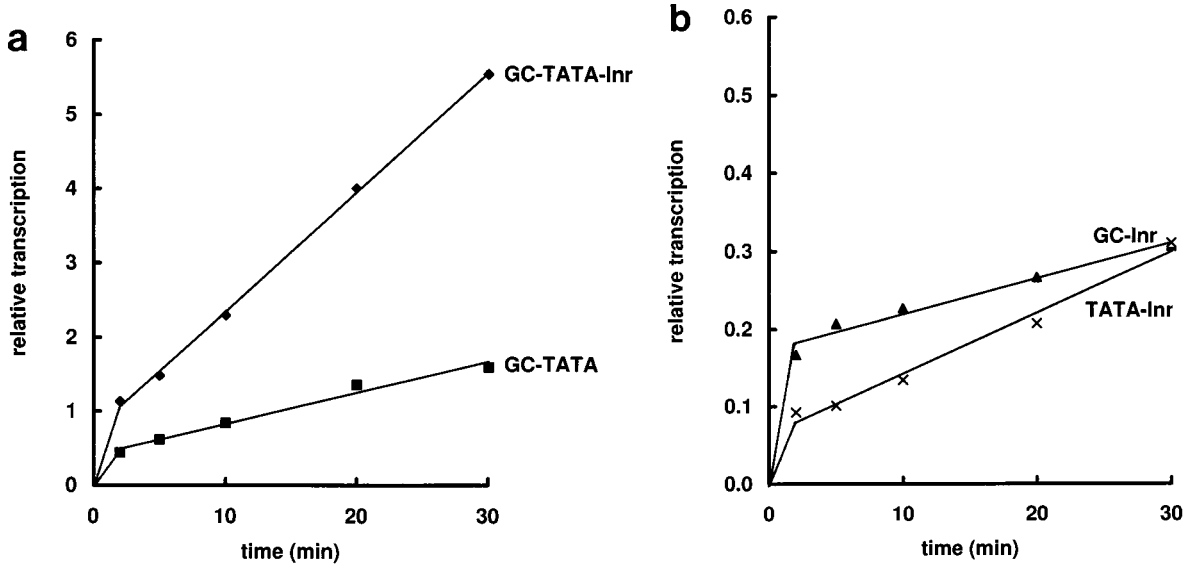

FIG. 3. Standard analysis of data from experiments of the type whose results are shown in Fig. 2. The cDNA products shown were quantified with a PhosphorImager and normalized to the amount of DNA present, which was in the range of 15 to 25 ng of supercoiled plasmid. The relative transcript level was plotted to generate the best-fit curves. Panels a and b are normalized to the same maximal transcription.

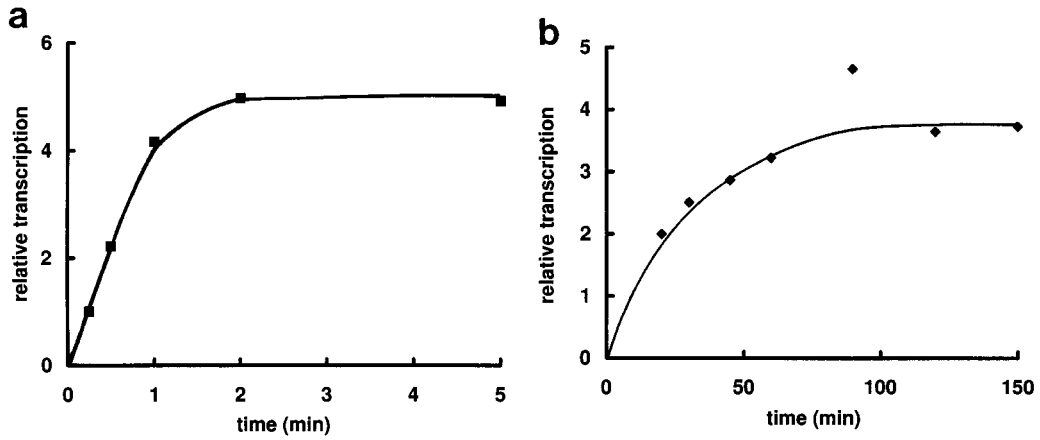

FIG. 4. Time to form and initiate GC-inr preinitiation complexes. Template and extract were incubated for 60 min, and then NTPs were added for the times indicated. (A) The amount of RNA produced in the following 5 min is shown, illustrating that initial transcription, including promoter clearance, is complete within 2 min. The other promoters studied showed completion within 1 minute. (B) The preincubation was done for the various times indicated, followed by the 2-min pulse with NTPs. The formation of preinitiation complexes is half complete in approximately 20 min under these conditions, and half completion typically requires 15 to 25 min for the various promoters used in this study.

analysis yields a continuous transcription rate of approximately 7 min per round (half time of 3.5 min). From prior work, the half time for formation of a preinitiation complex is known to be approximately 20 ± 5 min for this GC-TATA-inr promoter and some other promoters (15, 39, 40) (Fig. 4b and data not shown); the current data show that the 3.5-min half time for the subsequent continuous reinitiation process is four to seven times faster.

This very rapid reinitiation occurs on a template containing very strong activator and basal elements (six GC boxes and consensus TATA and TdT inr elements). It is possible that the rate of reinitiation would be different with other templates. Thus, we eliminated each of these three elements individually from the promoter and repeated the experimental analysis with the three derivative promoters. The plasmids are otherwise identical, minimizing potentially complicating effects of promoter clearance or elongation. Each template has an identical transcribed region, allowing direct comparisons of the amounts of RNA produced to be made (Fig. 2).

Figure 3 shows the analysis of the experiment for the parent and the three derivative templates: GC-TATA-inr, GC-TATA, GC-inr, and TATA-inr. In each case, the curve has the expected biphasic shape: an initial burst corresponding to synchronous first-round synthesis followed by a linear phase corresponding to continuous transcription. The data were analyzed as described for the parent.

The results are collected in Table 1, data set 1. Both the reinitiation half time and the level of active preinitiation complex formation are shown for the four templates, the latter normalized to that of the GC-TATA-inr promoter. The results show that reinitiation half times are shortened for the three promoters containing a consensus TATA box element; these show half times of 3.5 to 5 min compared to the preinitiation complex formation half time of approximately 20 min (15, 39, 40) (Fig. 4b and data not shown). By contrast, the TATA-less promoter shows a reinitiation half time of 19 min.

These experiments were repeated with a set of promoters in which the six GC boxes were replaced by five sites for the activator GAL-AH. The data were analyzed as described above, and the results are collected in Table 1, data set 2. The data show that the TATA-containing promoters reinitiate with half times of 5 to 7 min, whereas the TATA-less promoter shows a reinitiation half time of 20 min.

The two sets of data are in good agreement regarding the role of promoter elements in specifying rapid reinitiation within this set of promoters. When a consensus TATA box is present, the half times range from 3.5 to 7 min (five promoters in Table 1, data sets 1 and 2). This pertains whether the activator is SP1 or GAL-AH or even not present. Two TATA-less promoters show half times of 19 and 20 min. This pertains to both GC-box- and GAL-AH-activated promoters. Overall, the data show that the TATA-less promoters do not reinitiate faster than they form initial complexes. By contrast, reinitiation is facilitated up to three- to sixfold on promoters containing consensus TATA elements.

The data suggest that activator and inr elements may play a modest role in facilitating reinitiation, but this is not definitive. That is, the reinitiation half times range only from 3.5 to 7 min for the six consensus TATA promoters, which differ in whether they delete either strong activator or TdT inr elements. By

TABLE 1. Reinitiation rates and active preinitiation complex levels at promoters with various elements[a]

Data set and construct	PIC level[b]	Reinitiation half time (min)
1		
GC_6-TATA-inr	100	3.5
GC_6-TATA	40	5
GC_6-inr	15	19
TATA-inr	6	5
2		
AH_5-TATA-inr	100	6
AH_5-TATA	30	7
AH_5-inr	6	20
3		
AH_5-TATA-inr	100	6
AH_2-TATA-inr	35	7
AH_1-TATA-inr	10	5

[a] Data are the averages of two to four determinations. The promoters contain combinations of a consensus TATA element, the TdT inr, and the indicated number of GC boxes or GAL-AH activation sites.

[b] The active preinitiation complex (PIC) levels are normalized to 100 in each of the three data sets.

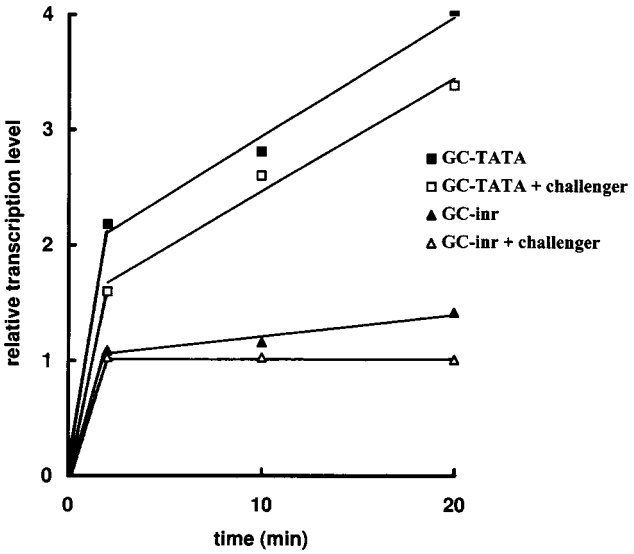

FIG. 5. Excess competitor does not interrupt continuous transcription on a TATA-containing template. Preinitiation complexes were formed during a 1-h incubation with 20 ng of GC-TATA or GC-inr templates. Then NTPs were added to all samples to begin synchronous initiation. At the same time, a 20-fold excess of a strong competitor (challenger) was added to two samples. The experiment was done in duplicate, and the average transcription level was plotted versus time after addition of NTPs. Later time points showed somewhat lower transcription levels for GC-TATA plus competitor. This experiment was repeated twice, and reproducible results were obtained.

contrast, it is clear that all three elements play important roles in forming active preinitiation complexes, as expected. The loss of various elements leads to reductions in complex levels ranging from 2.5- to 16-fold (Table 1, data sets 1 and 2; see PIC level).

As a further test of the role of activator in complex formation compared to reinitiation, we repeated the analysis with promoters containing either one, two, or five sites for the activator GAL-AH. The data show that increasing the number of active activator sites increases the number of active preinitiation complexes without detectably altering the reinitiation rate (Table 1, data set 3), as expected from the above considerations.

These data contribute to a modified view of the roles of elements in determining promoter strength in this series. For example (Fig. 3b), compare the GC-inr and TATA-inr promoters. The former is strong at early times, when preinitiation complex formation levels are critical, but not at later times. The switch is due to the buildup of RNA from more-rapid reinitiation in the promoter containing the consensus TATA box.

Competition experiments and facilitated reinitiation. The most likely source of rapid reinitiation is that after RNA polymerase initiates, factors remain associated with certain templates, and they assist in facilitating reinitiation (see the introduction for references). The dominant role of TATA shown above suggests that TATA may be required for such factors to stay behind. Prior studies showing that TFIID can be left behind were indeed done with promoters containing TATA boxes (27, 41). If factors are left behind and facilitate reinitiation, then the bound TATA template should continue to transcribe even when a large excess of competitor DNA is added.

To test this possibility, a challenge experiment was done. First, preinitiation complexes were formed at the GC-TATA promoter. Then nucleotides were added to begin synchronous initiation on this test template. At the same time, a 20-fold excess of a strong competitor (a GC-TATA-inr template with a truncated downstream region) was added. The amount of competitor is sufficient to nearly abolish transcription on the test promoter if competitor is added at the same time as the test template (not shown). Nonetheless, the data show that the test template continues to be transcribed for multiple rounds even though the excess competitor is present (Fig. 5). The slopes of the curves with and without competitor (the two upper curves) are indistinguishable for 20 min, showing that the competitor has not substantially interrupted the process of continuous RNA production. This result is consistent with earlier template commitment experiments done with a different TATA-containing template that showed that once the template is transcribed it is preferentially retranscribed (12, 35).

As a comparative control, this experiment was repeated, using a template which was identical except that the TATA box was replaced with an inr element. The result (Fig. 5, two lower curves) appears to be different. In this case, adding a competitor after initiation leads to a change in the slope of the curve, causing it to flatten (compare curves with triangles; seen in repeated experiments). Thus, the challenger has an inhibitory effect on the rate of continuous RNA production. However, because of the low rate of transcription on this TATA-less promoter, making the absolute differences in transcription even smaller, a quantitative analysis is difficult. The comparison with the TATA-containing template supports the view that the presence of a TATA box is important in directing factor commitment to a template in a way that leads to high rates of reinitiation.

A natural TATA-less promoter does not have facilitated reinitiation. Facilitated reinitiation has been shown to occur previously on a few templates containing TATA boxes in the context of natural basal promoter sequences (12, 14). Natural TATA-less templates have not been tested for reinitiation. We chose to study mouse DHFR, for which preinitiation complex formation has been studied extensively (10, 29). The promoter contains sites including GC boxes and an inr element but no TATA-like sequence. The assay used was the same as for Fig. 2 and 3.

The result is shown in Fig. 6. As before, an initial burst is followed by a linear increase in the amount of RNA. Analysis

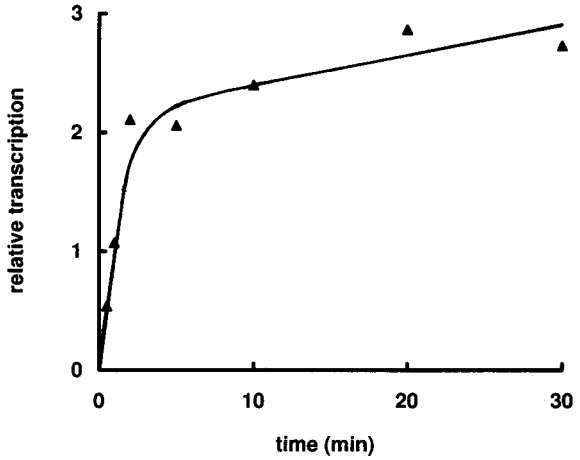

FIG. 6. The DHFR promoter has a low continuous transcription rate. A 20-ng sample of pSS625 containing the mouse DHFR promoter was studied according to the protocol described for Fig. 2 and 3. The half time for reinitiation is calculated to be 19 min, which is comparable to that for formation of preinitiation complexes (22 min).

TABLE 2. Effects of mutations in the consensus TATA element[a]

Construct and TATA sequence	PIC level[b]	Reinitiation half time (min)
Wild type (TATAAAA)	100	3.5
Mutant 1 (TTTAAAA)	100	5
Mutant 2 (TATTAAA)	55	5
Mutant 3 (TAAATAA)	60	7
Mutant 4 (TAAAAAA)	45	9
Mutant 5 (CATAAAA)	45	8

[a] The TATA sequences are listed in order from highest affinity for TBP to lowest according to Starr et al. (33) and are studied in the context of the GC-TATA-inr promoter. The data shown are the averages of three to six experiments.

[b] Active preinitiation complex (PIC) levels were normalized to that for the wild-type promoter, which was set at 100.

shows that the half time for reinitiation is approximately 19 min. The half time for assembly and promoter clearance of preinitiation complexes was found to be approximately 22 min (not shown), similar to those of several other promoters that have been assayed (15, 39, 40). Thus, the rates of initiation and reinitiation at this promoter are not significantly different, as predicted from the lack of a TATA box.

Effect of mutations in the consensus TATA box. These results demonstrate a critical need for a TATA box in directing rapid reinitiation at the promoters tested. As in many past experiments, the TATA element used had an optimal or nearly optimal sequence that is a close match to the consensus. The results of the competition experiments suggest that after initiation and the escape of the polymerase, factors stay on such TATA-containing templates to direct reinitiation. This is consistent with prior experiments that showed that the TATA-binding TFIID can be left behind on such templates (27, 41). It is possible that the use of suboptimal TATA sequences, which bind TFIID less tightly and exist in many natural promoters, would yield different results. As a preliminary test of this idea, we assayed templates containing mutant TATA boxes known to differ in both their affinity for TATA-binding protein (TBP) and their ability to direct transcription (33).

Five mutant TATA sequences were inserted separately in place of the consensus TATA element in the GC-TATA-inr promoter studied above. GC boxes and an inr element are included to enhance the signal, which is expected to be lower on the mutant templates. Each of the templates was assayed and analyzed as described above for the parent template. In addition, data were calculated from estimations of active preinitiation complex formation from 1- or 2-min pulses with NTPs and estimations of reinitiation rates by inclusion of 30-min pulses (without taking intermediate time points). The two kinds of analyses gave similar results (Table 2), which shows effects on both reinitiation rates and active preinitiation complex levels.

The data show that point mutations in the consensus TATA box lower the rate of reinitiation. The typical reduction is approximately twofold, increasing the half time from 3.5 min for the consensus to 5 to 9 min for the various mutants. All the point-mutated TATA boxes reinitiate faster than the 19 to 20 min observed for TATA-less promoters, which appear to reinitiate no faster than they form preinitiation complexes (see above). Thus, introduction of point mutations into the TATA box slows reinitiation but does not abolish the effect of the TATA element in making reinitiation faster than preinitiation complex formation.

DISCUSSION

Why do promoters have different combinations of elements, including many variants of the sequences of TATA boxes and inr elements? There have been many proposed answers to this question, and the current data suggest an additional important contributing factor. That is, such diversity may be important in specifying separate controls over when induction occurs and over how much RNA is made. The amount of RNA produced from a promoter in vivo should be primarily a consequence of the process of transcription reinitiation (14, 34). In the in vitro systems studied here, the sequence of the TATA box is critically important to the rate at which reinitiation occurs. Among the promoters studied, TATA-less promoters uniquely fail to show rapid, facilitated reinitiation. By contrast, all three elements studied, the TATA box, inr, and activator, contribute significantly to the induction of functional preinitiation complex formation.

These results apply to promoters that either do or do not have a consensus TATA box, a strong activator, and a TdT inr sequence. Deletion of TATA in this context drastically slows reinitiation, and the introduction of point mutations has an intermediate effect. The data also indicate that promoters with nonconsensus TATA sequences are weaker in vitro due to a combination of two effects: a reduced number of functional preinitiation complexes form, and this reduction is amplified by slower reinitiation from these complexes. Elimination of strong activator and TdT inr sequences leads principally to lower levels of functional preinitiation complex; it is possible, however, that these elements could play a role in reinitiation in the context of weaker promoters.

A likely source of the dominant effect of TATA on continuous transcription reinitiation rates is its ability to keep certain factors bound to the DNA after initiation and the escape of RNA polymerase during promoter clearance. A transcribing TATA-containing promoter resisted a challenge with excess competitor, showing that the transcribed template was preferentially retranscribed several times. Preferential retranscription of TATA templates has been shown previously (12, 35). The retention of TFIID was subsequently confirmed by direct experiments with TATA templates (27, 41).

Rapid reinitiation with several promoter constructs containing good matches to the TATA consensus has now been reported (reference 14 and above data) but was reported not to occur with a promoter that matches the consensus poorly (16). Table 2 shows that point mutations that reduce the affinity of TBP for TATA and reduce transcription (33) also lower the rate of transcription reinitiation. It has been shown previously that prebinding of TFIID and TFIIA to a TATA promoter can overcome a rate-limiting step and allow rapid transcription (37). Thus, the data suggest that TATA helps to bind factors including TFIID (27, 35, 41; see reference 19) and keeps them on the template after polymerase begins transcription, facilitating rapid reinitiation and high rates of continuous RNA production (14, 34). An analogous retention of factors after initiation has been known for some time for RNA polymerase III transcription (see reference 17).

In the system studied here, activator and inr sequences play a lesser role in promoting rapid reinitiation, but such effects could conceivably be greater at other promoters. However, when individual cells are assayed, activators appear to determine primarily the on-off function rather than the rate of RNA production. This has been indicated for both RNA polymerases I (25) and II (36). There is evidence that in some in vitro cases the activator is required for reinitiation of transcription (2, 22, 27, 39) but may not affect significantly the rate at which

this happens (1, 39). These observations are consistent with the postulated lesser role of activators in setting the rate of reinitiation, as opposed to a major role in induction. Thus, it is possible that certain activated TATA-less promoters may be easy to induce in vivo but might have the potential to be kept to low RNA production due to a lack of rapid reinitiation.

These considerations expand our understanding of why promoters are rarely built with simple consensus TATA and inr elements but exhibit diverse matches to both. The pathways for formation of preinitiation complexes are believed to be somewhat different depending on which basal elements are present and what their sequences are (3, 18, 42). There are major differences in how TATA and TATA-less inr promoters are repressed (6, 20, 21, 26, 28). There are also significant differences in activation (9, 23, 38). Many TATA-less promoters are involved in cell growth, differentiation, and development control and are highly regulated (4, 31). It is interesting that the current data raise the possibility that TATA-less promoters may be easier to rapidly down-regulate since reinitiation may be more easily interrupted (Fig. 5).

The current data add a new level to suggestions concerning why promoters are built to contain a variety of nonconsensus promoter elements. The specific sequences associated with each of the elements may make different contributions to two potentially independent properties of the promoter: how sensitive the promoter is to induction and how much RNA is made from the induced promoter. This proposal should stimulate further experimental tests of its applicability to natural promoters and of the mechanisms that underlie these controls of promoter functions.

ACKNOWLEDGMENTS

We are very grateful to Branden Wolner for constructing the truncated GC-TATA-inr plasmid, to Ming Yan and P. Farnham for advice on DHFR transcription, to Yi Song for technical assistance, and to members of the Gralla laboratory and Steve Smale for helpful advice.

This work was supported by NIH grant GM49048 and by a predoctoral traineeship to D.Y.(GM07185).

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