Kinetic Analysis of Sp1-Mediated Transcriptional Activation of a TATA-Containing Promoter†

Satya Narayan‡ and Samuel H. Wilson*,§

Sealy Center for Molecular Science and Sealy Center for Oncology and Hematology, University of Texas Medical Branch, Galveston, Texas 77555, and Laboratory of Structural Biology, National Institutes of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

Received June 3, 1999; Revised Manuscript Received October 5, 1999

ABSTRACT: Using a HeLa cell nuclear extract (NE)-based in vitro runoff transcription system, we have examined the effect of Sp1 on the activation of a TATA-containing chimeric DNA polymerase β (pAS8) promoter. The results demonstrated that the TATA element-dependent basal activity of the pAS8 promoter was stimulated 4-fold by supplementation of a Sp1-depleted HeLa cell nuclear extract (NEd) with purified human Sp1, indicating that pAS8 promoter activity is dependent upon Sp1. A detailed kinetic analysis based on a three-step kinetic model of transcription initiation showed that Sp1 stimulates the activity of the pAS8 promoter by increasing the amount of closed preinitiation complex (RP_c) assembly as well as by enhancing the rate of promoter clearance (k_3). There was no significant effect of Sp1 on the apparent rate of open complex (RP_o) formation (k_2) of the pAS8 promoter. These studies define more precisely the kinetic mechanisms by which Sp1 may regulate the rate of transcript formation of a TATA-containing promoter.

Gene expression in eukaryotic cells is regulated in part at the level of transcriptional initiation, which involves a series of site-specific protein·DNA and protein·protein interactions (*1*−*3*). To begin RNA synthesis by RNA polymerase II (RNA pol II)[1] at the transcription initiation site, several basal factors and RNA pol II itself bind to a promoter in a multistep process to form a preinitiation complex (*4*). In TATA-containing promoters, TFIID binds first to a TATA-core element to nucleate the transcription initiation site, followed by the binding of a series of basal factors in a highly ordered fashion (*3*).

Regulated expression of genes utilizing the basal transcription apparatus is achieved using several upstream activators that participate in the transcription initiation process (*5*). Transcriptional initiation is a complex process, which consists of three distinct steps, as described by McClure with regard to prokaryotic gene regulation (*6*). Step I of the transcriptional initiation process is described for assembly of transcription factors on the promoter at the transcription start site to form a closed preinitiation complex (RP_c). Step II is an ATP-dependent isomerization of DNA template to form an open preinitiation complex (RP_o), and step III is promoter clearance. Activator proteins may regulate a target gene's expression by influencing any one or more of these steps (*7*−*13*).

The transcriptional activator Sp1 is a member of the zinc finger family of transcription factors and binds to DNA at a consensus GC-box sequence (*14*). There are three other Sp1-related proteins, known as Sp2, Sp3, and Sp4 (*15, 16*). The Sp1 family members are ubiquitously expressed. These factors are glutamine- and serine/threonine-rich proteins that have similar DNA-binding specificity, but display differential effects on gene regulation (*17*−*19*). Among these, Sp1 is the best studied factor, and is known to activate many GC-box-containing viral and cellular promoters (*20*−*23*). Sp1 interacts with the transcription preinitiation complex through the TFIID-complex with specific binding to TATA-associated factors (TAF) (*24*).

It has been suggested that activators may enhance the rate of transcription by enhancing recruitment of basal transcription factors onto the promoter and by altering the conformation of prebound factors, thus modulating their activator or suppressor activity (*7*). In a recent study, Yean and Gralla (*13*) examined the kinetics of RP_c assembly and showed that the GC element of the terminal deoxynucleotidyl transferase gene (TdT; a TATA-containing promoter) does not activate the apparent rate constant for RP_c assembly (k_1). They showed that Sp1 stabilized the binding of an essential factor, which may have enhanced the stability of the prebound factors, producing increased numbers of functional RP_c complexes. Although transcription initiation is a multistep

† This work was supported by NIH Grants CA77721 (to S.N.) and ES06492 (to S.H.W.).

* Address correspondence to this author at the Laboratory of Structural Biology, National Institutes of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709. Tel.: 919-541-3267, FAX: 919-541-3592, Email: Wilson5@NIEHS.NIH.Gov.

‡ University of Texas Medical Branch.

§ National Institutes of Environmental Health Sciences.

[1] Abbreviations: β-pol, DNA polymerase β; Inr, initiator; MLP, adenovirus major late promoter; NE, HeLa cell nuclear extract; NEd, Sp1-depleted NE; RNA pol II, RNA polymerase II; RP_c, closed preinitiation complex; RP_o, open preinitiation complex.

10.1021/bi9912701 CCC: $19.00
Published on Web 01/07/2000

process, the observations derived from these studies were limited to step I of transcription initiation, i.e., RP_c assembly. Whether the increased amount of Sp1-mediated transcript formation was also partially mediated by the enhanced apparent rate of RP_o formation (k_2) and/or an enhanced rate of promoter clearance (k_3) is not known.

In the present investigation, we used a three-step kinetic model of transcription initiation to determine the quantitative effect of Sp1 on the activation of a TATA-containing chimeric DNA polymerase β (β-pol) promoter (pAS8). The results discussed in this paper provide a basis for understanding and evaluating the effect of Sp1 on the kinetic parameters of the transcription initiation of a TATA-containing promoter.

MATERIALS AND METHODS

Plasmids. The nucleotide sequences of the pAS8 promoter have been derived from a TIS-CAT construct in which the far distal Sp1 site was removed, leaving one distal Sp1 site (*9*). The proximal Sp1 site of the TIS-CAT was replaced with a TATA element, and the β-pol promoter initiation site of the TIS-CAT was replaced with the adenovirus major late promoter (MLP) initiation site (*9, 15*). The pAH1 construct (derived from the pAS8 promoter) serves as a control in which the remaining Sp1 site of the pAS8 promoter was deleted. The 5′ ends of these promoters were linked to a vector with the *Hin*dIII site, and the 3′ ends were joined to CAT-reporter gene coding sequences. The detailed structures of these promoters are shown in Figure 1.

In Vitro Runoff Transcription Assay. HeLa cell (S3, from ATCC) nuclear extract was prepared by the procedure of Shapiro et al. (*26*). Sp1-depleted NE (NEd) was prepared as described by Jackson and Tjian (*27*). Human recombinant Sp1 was obtained from Promega. Plasmid DNA substrates were linearized with *Pvu*II to generate a 180 nt runoff transcript with pAS8 and pAH1 promoters. In vitro transcription reactions were carried out at 25 °C in a final volume of 25 μL containing 20 mM Hepes (pH 7.9), 2 mM DTT, 6.5 mM $MgCl_2$, 65 mM KCl, 10% glycerol (v/v), 20 units of RNasin (Promega), and 30 μg of NE, NEd, or NEd supplemented with Sp1. One microgram of plasmid DNA was added to the reaction mixture and preincubated for 30−45 min to form closed preinitiation complexes (RP_c). After preincubation, transcription was initiated by addition of ATP, GTP, UTP (500 μM each), CTP (25 μM), and 10 μCi of [α-^{32}P]CTP (800 Ci/mmol, DuPont, NEN). Sarkosyl was added (0.25%, w/v) 2 min after the addition of NTPs, to limit the reaction to single-cycle transcript formation by preventing reinitiation events. Transcription was stopped after the indicated period with a solution containing 0.2 M Tris-HCl (pH 7.5), 25 mM EDTA (pH 8), 2% SDS (w/v), and 15 μg of torula yeast RNA (*9*). Deviation from (or modification of) these transcription conditions is indicated in the figure legends. Runoff transcripts were separated on a 6% polyacrylamide/8 M urea gel, which was dried and exposed to X-ray film. Autoradiograms were quantified with an Imager (Appligene) and analyzed by VISAGE electrophoresis gel analysis system (Millipore).

A three-step kinetic model (*6, 9, 10*) was used to determine the effects of Sp1 on each of the three stages of transcription initiation, i.e., RP_c assembly, RP_o formation and promoter clearance. A general scheme for this minimum transcription initiation model is given below:

$$R + P \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} RP_c \xrightarrow{k_2} RP_o \xrightarrow{k_3} \text{Transcript}$$

$$K_B \qquad \text{NTPs}$$

$$\text{Step I} \quad \text{Step II} \quad \text{Step III}$$

where R = RNA pol II, P = promoter, K_B = equilibrium association constant, k_1 = association rate constant, k_{-1} = dissociation rate constant, k_2 = rate constant for open complex formation, and k_3 = rate constant for promoter clearance.

A quantitative analysis of the influence of Sp1 on the rate constants of transcription initiation was carried out as described previously for the ATF/CREB-dependent activation of the pSH15 promoter (*9, 10*). Data analyses and presentations were carried out with Kaleidagraph (Synergy Software) or Sigma Plot (Jandel Scientific).

$KMnO_4$ Footprinting of Open Preinitiation Complex (RP_o) Formation. The $KMnO_4$ footprinting assay for RP_o formation was performed as described earlier (*9*−*11*). Briefly, the in vitro runoff transcription assay with pAS8 plasmid, NEd, or NEd supplemented with purified Sp1 was set up as described above. After a 30 min preincubation at 25 °C to form RP_c, 400 μM dATP was added and preincubation was continued for different periods. A 6 mM $KMnO_4$ solution was then added to each tube, and the reaction mixture was incubated for 4 min at 37 °C. The DNA was purified, and the $KMnO_4$-modified T residue at the +1 position of the "transcribed strand" of the pAS8 promoter was analyzed by primer extension using DNA polymerase I (Klenow fragment). The selection of primer and characterization of the $KMnO_4$ footprinting assay were the same as described earlier (*9, 10*).

RESULTS

Transcriptional Activation of pAS8 Promoter Is Dependent upon Sp1. In these studies, we used pAS8, a chimeric β-pol promoter, to examine the effects of Sp1 on the kinetic parameters of transcription initiation of a TATA-containing promoter. The pAS8 promoter was constructed from a TIS-CAT promoter (*9*), which contains a TATA-box and an initiation site derived from the adenovirus MLP and a single Sp1-binding site. The control plasmid, pAH1, lacks the Sp1-binding site, but retains the MLP-derived TATA-box and initiation site (Figure 1). A HeLa cell nuclear extract-based in vitro transcription assay system was used in these studies. For some experiments, the nuclear extract was depleted of Sp1 as described by Jackson and Tjian (*27*). In these preparations, more than 90% of the Sp1 protein was removed from the NE, as determined by gel-shift analysis (data not shown).

To examine the effect of Sp1 on the transcriptional activation of the pAS8 promoter, in vitro transcription was carried out with NE, NEd (Sp1-depleted NE), or NEd supplemented with purified Sp1. Synthesis of the runoff transcript was greater from pAS8 using NE as a source of transcription factors than when using NEd (Figure 2A, lanes 1 and 2). The pAH1 promoter, on the other hand, has a similar (and lower) level of activity with either NE or NEd (Figure 2A, lanes 3 and 4), which is consistent with the basal level of transcription observed with TATA-box-containing

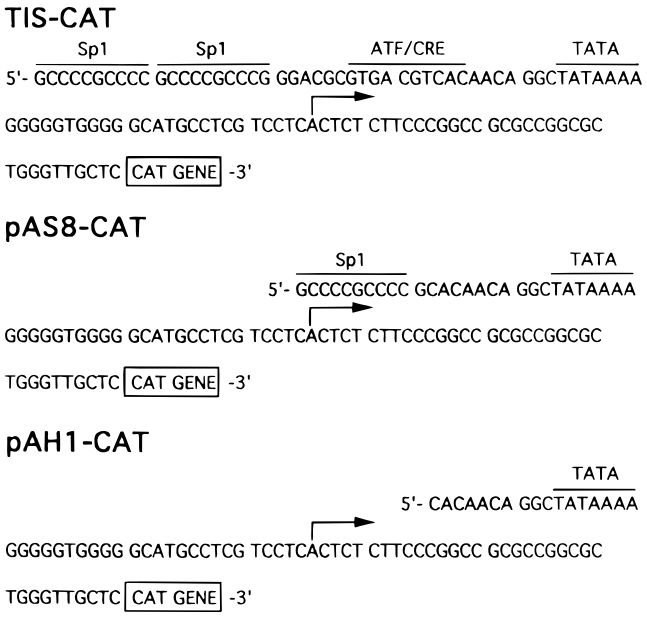

FIGURE 1: Structures of the CAT-reporter constructs. Nucleotide sequences of the promoter constructs pAS8 and pAH1 are given, which are derived from TIS-CAT, a chimeric construct of the β-pol promoter. The transcription start sites are denoted with arrows.

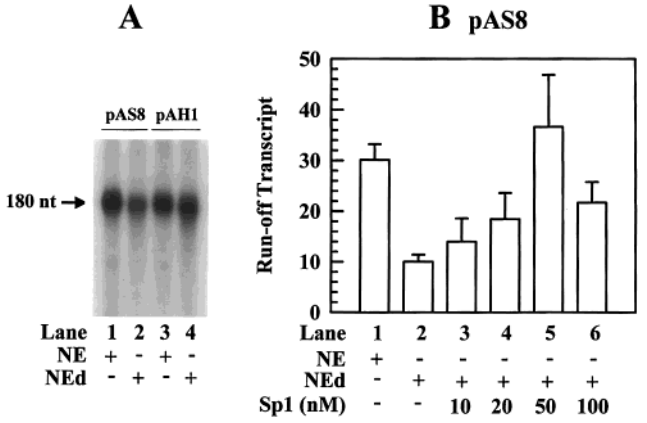

FIGURE 2: Transcriptional activation of pAS8 promoter by Sp1. Panel A: pAS8 and pAH1 promoter activity with NE (lanes 1 and 3) or NEd (lanes 2 and 4), respectively, is shown. Arrow denotes location of 180 nt runoff transcript. Panel B: To examine the effects of Sp1 on the transcriptional activity of the pAS8 promoter, the transcription assay was assembled with increasing amounts of recombinant human Sp1 (lanes 3−6: 10, 20, 50, and 100 nM, respectively). The runoff transcript levels were quantified and plotted in arbitrary densitometric units. Data are mean ± SE of three independent experiments.

promoters (*9, 28*). This indicates that Sp1 is required for maximal activity of the pAS8 promoter. To examine whether the decreased activity of the pAS8 promoter using NEd was due to lack of Sp1, we performed transcription assays where purified Sp1 was added to the NEd and the runoff transcript was measured. The results showed that the addition of purified Sp1 to NEd restored the decreased pAS8 promoter activity in a dose-dependent manner (Figure 2B, lanes 3−6). These results indicate that activation of pAS8 promoter is dependent upon Sp1. After addition of 50 nM Sp1, there was a consistent 4-fold increase in pAS8 promoter activity (Figure 2B, lane 5). The pAS8 promoter activity with 100 nM Sp1 was lower (Figure 2B, lane 6) than with 50 nM Sp1 (Figure 2B, lane 5), which may be due to a squelching effect of Sp1 at higher concentrations on the transcriptional initiation of the pAS8 promoter. Based on these results, a concentration of 50 nM Sp1 was used in the future experiments.

Apparent Rate Constant for RP_c Assembly (k_1) of pAS8 Promoter Is Not Affected by Sp1, but the Formation of Functional RP_c Is Increased. In a purified reconstituted transcription system, it has been reported that activators bind to their consensus DNA sequences and then participate in preinitiation complex assembly by interacting with basal transcription factors (*7, 11, 12, 24, 29, 30*). Since RP_c assembly is a slow process (*9, 31*), it is possible that the Sp1 could enhance the recruitment of factors on the promoter and increase the amount of transcript formation.

To examine these possibilities, the kinetic parameters of Sp1-mediated RP_c formation were examined using the following transcription conditions. The TATA-containing promoter on pAS8 was preincubated with NEd or with NEd supplemented with 50 nM Sp1. After different periods, an aliquot of the preincubation mixture was removed, and transcription was initiated with NTPs. To determine the amount of RP_c formed, a single-cycle runoff transcript reaction was carried out, and the amount of product was measured (Figure 3). The data were fitted to a single binding model to determine the apparent rate constant for RP_c assembly (k_1) and the amount of RP_c formed (*9*). The results indicate that Sp1 did not significantly change the apparent rate of RP_c assembly on the pAS8 promoter (Figure 3C, compare promoter activity with and without Sp1). However, a 3-fold increase in the amount of RP_c formation was observed when transcription reactions with pAS8 were carried out in the presence of NEd supplemented with 50 nM Sp1 versus NEd alone (Figure 3C). These results suggest that Sp1 increased the amount of RP_c assembly on the pAS8 promoter without changing the apparent rate of recruitment (k_1). Similar results indicating that a TATA-containing promoter increased Sp1-mediated recruitment (amount) of RP_c were reported previously (*13*).

Apparent Rate Constant (k_2) for Open Complex Formation (RP_o) of pAS8 Promoter Is Unchanged by Sp1. After establishing that Sp1 enhances the amount of RP_c recruitment onto the pAS8 promoter, we further examined its effects on the succeeding steps of transcription initiation, i.e., the apparent rate of open complex formation (k_2) and/or promoter clearance (k_3). In prokaryotic systems, it is well documented that activator proteins regulate gene expression by affecting the binding constant (K_B), k_2, or k_3 (*6, 32−34*). Recently, in eukaryotic systems we have used a similar kinetic model to describe the role of an ATF/CREB protein in the transcriptional regulation of the pSH15 promoter (*9, 10*). The experimental approach used in the present study was similar to that utilized for the study of ATF/CREB. A time course for transcript formation was generated with pAS8 and NEd or with NEd supplemented with 50 nM Sp1. In this protocol, the nuclear extracts, plasmids, and NTPs were added at '0' time. At different time points, aliquots were removed from the preincubation mixture, and runoff transcripts were measured. To determine k_2, the data were fitted to an equation which describes a lag phase followed by a linear phase using nonlinear least-squares methods (*9*). A distinct lag in runoff transcript formation with pAS8 promoter was observed, followed by a steady-state rate of transcript formation (V_{ss}) (Figure 4A). The observed k_2 for the pAS8 promoter with

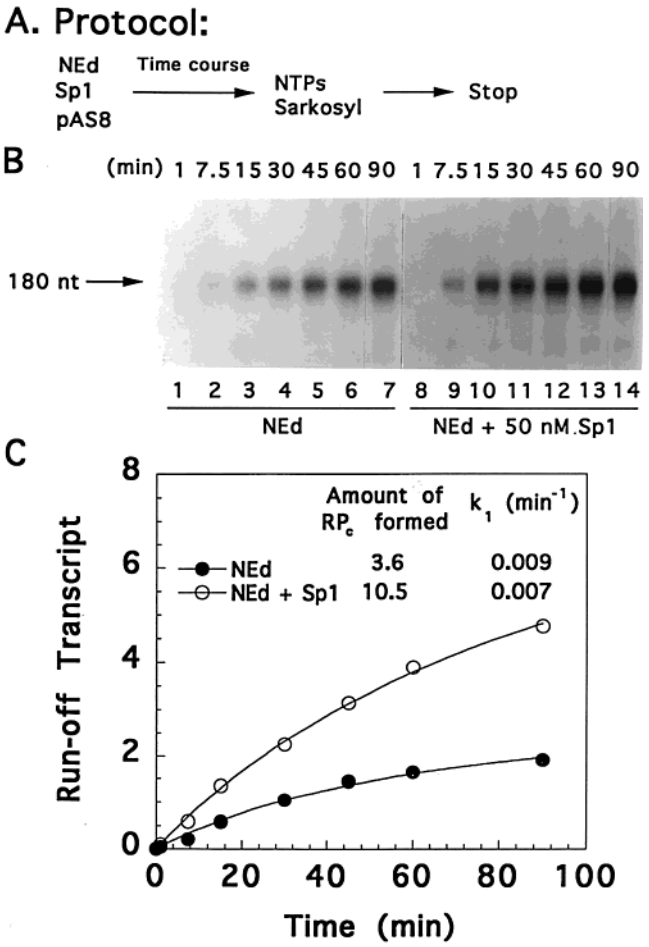

FIGURE 3: Sp1-mediated recruitment of RP_c is increased on pAS8 promoter. Panel A: Sp1-mediated assembly of RP_c was examined by incubating the pAS8 plasmid with NEd plus/minus 50 nM Sp1 (Protocol). Panel B: An autoradiograph of a time course experiment. At different time points, aliquots of the reaction mixture were removed, NTPs were added, and single-cycle runoff transcription was carried out in the presence of 0.25% (w/v) sarkosyl. Panel C: The data from panel B were quantified and fitted into computer-derived theoretical hyperbolic curves. Data shown are arbitrary densitometric units from two different experiments.

NEd (0.11 min^{-1}; without Sp1) or with NEd supplemented with Sp1 (0.09 min^{-1}) was similar.

To further examine whether Sp1 affects k_2, we performed $KMnO_4$ footprinting of RP_o formation. In this assay system, $KMnO_4$ reacts selectively with a T residue in single-stranded DNA and produces hypersensitivity at the T residue after primer extension with Klenow fragment (*11*). In the previous studies, we have characterized this technique by measuring hypersensitivity of the +1T residue of the "transcribed strand" as a function of RP_o formation (*9, 10*). The reaction mixture was assembled for RP_c formation with pAS8 plasmid, NEd, or NEd supplemented with 50 nM Sp1. Then dATP was added, and the incubation was continued for different periods. Although the $t_{1/2}$ of RP_o formation is about 2.5 min (*11*), we incubated for longer periods (10 min) to convert most of the RP_c to RP_o, and then probed with $KMnO_4$. The results are shown in Figure 4B. When measured as a function of time after dATP addition, we observed no significant difference in the appearance of $KMnO_4$ hypersensitivity at the +1T residue in the absence (lanes 1−5) or presence of 50 nM Sp1 (lanes 6−10). Thus, the $KMnO_4$ footprinting results are consistent with the kinetic results

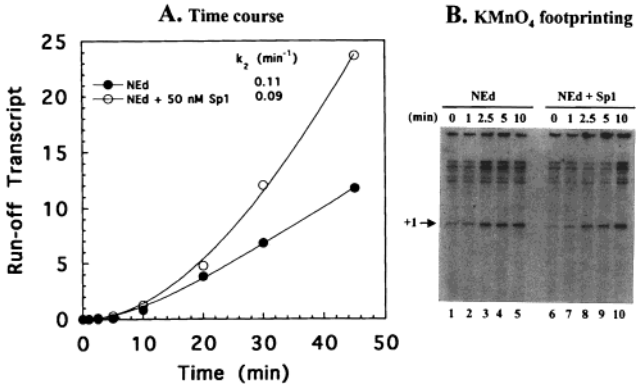

FIGURE 4: Effect of Sp1 on the rate of open complex formation (k_2) of pAS8 promoter. Panel A: Time course of transcript formation. Transcript formation was measured at various time intervals with pAS8 promoter in the presence of NEd plus/minus 50 nM Sp1. NTPs were added to initiate the reaction at '0' time. Runoff transcripts were quantified in arbitrary densitometric units. The solid lines represent computer-derived theoretical curves based upon the best fit of the data to a mathematical equation as described earlier (*9*). The kinetic parameters lag (τ_{obs}, relaxation time required for RP_c to RP_o transition $= 1/k_2$) and the steady-state rate of transcript formation (V_{ss}, runoff transcript/min $= [RP_o]k_3$) were determined as described (*9, 10*). Panel B: $KMnO_4$ footprinting for Sp1-dependent opening of the initiation site of pAS8 promoter during RP_o formation. The RP_c was assembled with pAS8 plasmid and NEd (lanes 1−5) or with NEd supplemented with 50 nM Sp1 (lanes 6−10) for 30 min. Then 400 μM dATP was added, and incubation was continued for different periods before probing with $KMnO_4$. The dATP incubation time is given on the top of each panel. The hypersensitive T residue at the +1 position is shown by the arrow.

described above, suggesting that Sp1 does not affect k_2 of the pAS8 promoter (Figure 2A).

Although Sp1 did not affect k_2 for the pAS8 promoter, the apparent steady-state rate of transcript formation (V_{ss}) was significantly increased for both (Figure 4A). Since V_{ss} is a function of $[RP_o]$ and k_3, and k_2 did not contribute to the observed increase in Sp1-mediated transcript formation, then Sp1 may increase V_{ss}, either by increasing $[RP_o]$ or by increasing k_3, or both. It is expected that the amount of RP_o will be similar to the amount of RP_c assembled on both types of promoters. A similar observation was found in our previous studies with the pSH15 promoter (an ATF/CREB-dependent TATA-containing chimeric promoter). In those studies, the amounts of RP_c and RP_o were increased by purified ATF/CREB protein from MNNG-treated HeLa cells, while no increase in k_2 occurred (*10*). Thus, in the present studies, the most likely explanation for the increased V_{ss} would be an increase in k_3.

Effect of Sp1 on the Apparent Rate of Promoter Clearance (k_3) of pAS8 Promoter. To examine the effects of Sp1 on k_3 of the pAS8 promoter, a time course of transcript formation was generated starting from RP_o (*9, 10*). RP_c was assembled with NEd for 30 min, and then dATP was added to form RP_o. Transcription was initiated with preassembled RP_o by adding NTPs and incubating plus/minus 50 nM Sp1 (Figure 5A). The amount of 180 nt runoff transcript was measured after different periods (Figure 5B). Since Sp1 was added to the reaction mixture after RP_o was formed, any Sp1-mediated difference in the rate of transcript formation will be a function of k_3, and k_2 is insensitive to Sp1. The results indicate that the rate of pAS8 promoter clearance was 3-fold higher in

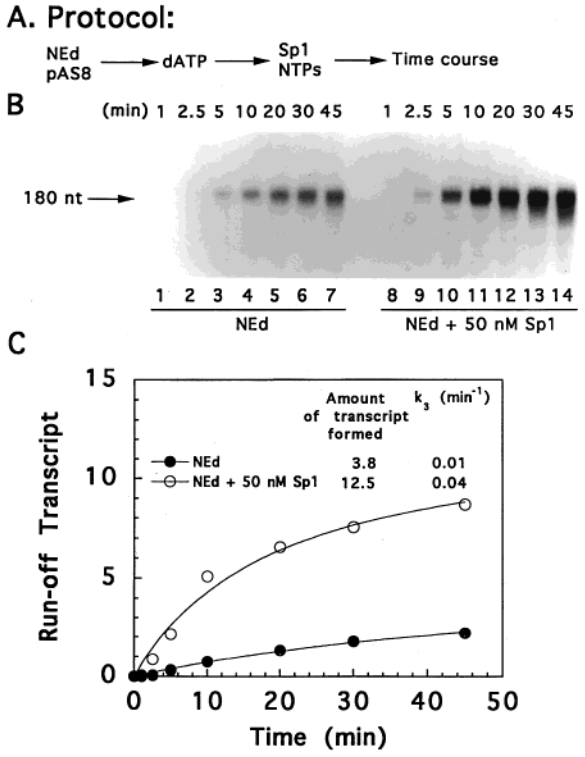

FIGURE 5: Sp1-mediated activation of the apparent rate of promoter clearance (k_3) of the pAS8 promoter. The protocol for k_3 determination is given in panel A. RP_c was assembled with pAS8 plasmid and NEd plus/minus 50 nM Sp1. After 30 min preincubation, 400 μM dATP was added to the transcription mixture, and preincubation was continued for an additional 5 min to allow the conversion of RP_c into RP_o. Sp1 and NTPs were then added to the transcription mixture, and a time course of transcript formation was determined. A representative autoradiogram is shown in panel B. The data were fitted into computer-derived curves (panel C) to determine k_3. Data are derived from two independent experiments.

the presence of NEd supplemented with Sp1 than in the presence of NEd alone (Figure 5C). Since k_3 is also a function of V_{ss}/[RNA pol II] and the concentration of RNA pol II in our assay system is approximately 1.5 nM, the rate of transcript formation per minute (V_{ss}) with NEd or NEd supplemented with 50 nM Sp1 will be 0.015 and 0.060 nM, respectively. The Sp1-mediated increase in k_3 of the pAS8 promoter was similar, and also corresponded to the amount of RP_c. These results suggest that RP_c assembled with pAS8 is strongly stabilized by Sp1, which leads to a quantitative increase in promoter clearance.

DISCUSSION

In the present investigation, the mechanism of Sp1 action and its influence on kinetic parameters of a TATA-containing chimeric β-pol promoter (pAS8) were examined. For TATA-less promoters, it has been suggested that Sp1 interacts with the TFIID complex via a heat-labile tethering factor (*35*, *36*). On the other hand, in most of the TATA-containing promoters, the TFIID complex nucleates the transcription initiation site (*3*). A similar effect was observed on the Sp1-mediated increase in the recruitment of RP_c on the pAS8 promoter (Figures 2 and 3). The rate constant for RP_c assembly by Sp1 was unchanged for pAS8 promoter. Our results on Sp1-mediated recruitment of RP_c and the rate of RP_c assembly of the pAS8 promoter are in agreement with those reported by Yean and Gralla (*13*). Under our experimental conditions, the presence of Sp1 may be required during preincubation to have an effect on the recruitment of RP_c. This is consistent with previous reports where the interaction of Sp1 with basal factors is required to stimulate transcription (*24*, *35*, *37*−*40*).

ATF/CREB, in its phosphorylated form, is a transcriptional activator protein which has been shown to influence the overall rate of transcript formation of a TATA-containing promoter (pSH15) by influencing more than one step of the transcription initiation process (*10*). Therefore, in this study, potential roles for Sp1 at all stages of transcription initiation were examined. Our results suggest that Sp1 did not influence the rate of open complex formation (k_2) with pAS8 promoter. Furthermore, the rate of promoter clearance (k_3) of the pAS8 promoter was increased by Sp1, suggesting that the overall rate of transcript formation of a TATA-containing promoter is regulated by Sp1 both by increasing the amount of RP_c assembled and by stimulating the rate of promoter clearance. The latter effect of Sp1 on a TATA-containing promoter is similar to that of ATF/CREB (*10*). Thus, activators may influence the rate of transcript formation of TATA-containing promoters at multiple steps of the transcription initiation process.

The involvement of activator proteins at one or more steps of transcription initiation can be explained by their interactions with different basal factors. For example, when Sp1 is present during preincubation, it may recruit and stabilize basal factors through its interaction with the TAF_{110} and TAF_{55} subunits of the TFIID complex (*39*, *40*). Furthermore, the TFIID complex also interacts with the TFIIE and TFIIH complexes, which may be involved in the regulation of gene transcription at the step of promoter clearance (*39*, *41*). However, when Sp1 enters into the transcription initiation process after RP_o is formed (or remains present during RP_c assembly but is activated after RP_o is formed) of TATA-containing promoters where all these basal factors are present in the RP_c, it may influence the activity of the preexisting factors by modulating their protein·protein interactions and stimulating promoter clearance. These possibilities can be tested with a purified reconstituted transcription system.

In summary, we have provided a simple three-step kinetic model, and compared the kinetic parameters of transcription initiation of a TATA-containing promoter in the presence and absence of Sp1. Thus, the effect of the transcription activator Sp1 on these parameters has been defined, to better understand the mechanism by which Sp1 modulates the rate of transcript formation.

ACKNOWLEDGMENT

We thank Drs. David Konkel and Miriam Sander for critically reviewing the manuscript.

REFERENCES

1. Lewin, B. (1990) *Cell 61*, 1161−1164.
2. Van Dyke, M. W., Roeder, R. G., and Sawadogo, M. (1988) *Science 241*, 1335−1338.
3. Tang, H., Sun, X., Reinberg, D., and Ebright, R. H. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 1119−1124.
4. Zawel, I., and Reinberg, D. (1993) *Prog. Nucleic Acid Res. Mol. Biol. 44*, 67−108.
5. Choy, B., and Green, M. R. (1993) *Nature 366*, 531−536.
6. McClure, W. R. (1985) *Annu. Rev. Biochem. 54*, 171−204.

7. Goodrich, J. A., Cutler, G., and Tjian, R. (1996) *Cell 84*, 825−830.
8. Hai, T., Horikoshi, M., Roeder, R. G., and Green, M. R. (1988) *Cell 54*, 1043−1051.
9. Narayan, S., Widen, S. G., Beard, W. A., and Wilson, S. H. (1994) *J. Biol. Chem. 269*, 12755−12763.
10. Narayan, S., Beard, W. A., and Wilson, S. H. (1995) *Biochemistry 34*, 73−80.
11. Wang, W., Carey, M., and Gralla, J. D. (1992) *Science 255*, 450−453.
12. Wang, W., Gralla, J. D., and Carey, M. (1992) *Genes Dev. 6*, 1716−1727.
13. Yean, D., and Gralla, J. D. (1996) *Nucleic Acids Res. 24*, 2723−2729.
14. Kadonaga, J. T., Carner, K. C., Masiarz, F. R., and Tjian, R. (1987) *Cell 51*, 1079−1090.
15. Hagen, G., Muller, S., Beato, M., and Suske, G. (1992) *Nucleic Acids Res. 20*, 5519−5525.
16. Kingsley, C., and Winoto, A. (1992) *Mol. Cell. Biol. 12*, 4251−4261.
17. Hagen, G., Muller, S., Beato, M., and Suske, G. (1994) *EMBO J. 13*, 3843−3851.
18. Dennig, J., Hagen, G., Beato, M., and Suske, G. (1995) *J. Biol. Chem. 270*, 12737−12744.
19. Hagen, G., Dennig, J., Preiss, A., Beato, M., and Suske, G. (1995) *J. Biol. Chem. 270*, 24989−24994.
20. Dynan, W. S., and Tjian, R. (1983) *Cell 32*, 669−680.
21. Dynan, W. S., and Tjian, R. (1983) *Cell 35*, 79−87.
22. Dynan, W. S., and Tjian, R. (1985) *Nature 316*, 774−778.
23. Gidoni, D., Kadonaga, J. T., Barerra-Saldana, H., Takahashi, K., Chambon, P., and Tjian, R. (1985) *Science 230*, 511−517.
24. Chen, J.-L., Attardi, L. D., Verrijzer, C. P., Yokomori, K., and Tjian, R. (1994) *Cell 79*, 93−105.
25. Widen, S. G., Kedar, P., and Wilson, S. H. (1988) *J. Biol. Chem. 263*, 16992−16998.
26. Shapiro, D. J., Sharp, P. A., Wahli, W. W., and Keller, M. J. (1988) *DNA 7*, 47−55.
27. Jackson, S. P., and Tjian, R. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 1781−1785.
28. Kaufman, J., and Smale, S. T. (1994) *Genes Dev. 8*, 821−829.
29. Goodrich, J. A., and Tjian, R. (1994) *Cell 77*, 145−156.
30. Leiberman, P. M., and Berk, A. J. (1994) *Genes Dev. 8*, 995−1006.
31. Wampler, S. L., and Kadonaga, J. T. (1992) *Genes Dev. 6*, 1542−1552.
32. Hawley, D. K., and McClure, W. R. (1982) *J. Mol. Biol. 157*, 493−525.
33. Hershberger, P. A., and de Haseth, P. L. (1991) *J. Mol. Biol. 222*, 479−494.
34. Goodrich, J. A., and McClure, W. R. (1992) *J. Mol. Biol. 224*, 15−29.
35. Smale, S. T., Schmidt, M. C., Berk, A. J., and Baltimore, D. (1990*) Proc. Natl. Acad. Sci. U.S.A. 87*, 4509−4513.
36. Pugh, B. F., and Tjian, R. (1991) *Genes Dev. 5*, 1935−1945.
37. Pugh, B. F., and Tjian, R. (1990) *Cell 61*, 1187−1197.
38. Hoey, T., Weinzierl, R. O. J., Gill, G., Chen, J.-T., Dynlacht, B. D., and Tjian, R. (1993) *Cell 72*, 247−270.
39. Gill, G., Pascal, E., Tseng, Z. H., and Tjian, R. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 192−196.
40. Chiang, C. M., and Roeder, R. G. (1995) *Science 267*, 531−536.
41. Maxon, M. E., Goodrich, J. A., and Tjian, R. (1994) *Genes Dev. 8*, 515−524.

BI9912701