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The GC Box and TATA Transcription Control Elements in the P38 Promoter of the Minute Virus of Mice Are Necessary and Sufficient for Transactivation by the Nonstructural Protein NS1

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To further define the transcriptional regulation of the P38 promoter in the minute virus of mice (MVM) genome, we constructed a series of internal deletion and linker scanning mutations. The mutant P38 constructs were assayed for transcriptional activity in vitro by primer extension analysis with nuclear extracts from murine A92L fibroblasts. Mutations which disrupted the GC box and TATA box severely reduced transcription in vitro. DNase I footprinting analysis confirmed that the murine transcription factor Sp1 bound to the GC box; however, no factors were observed interacting with a putative transcriptional activation regulatory element, termed the TAR element. The linker scanning mutations were analyzed in vivo by using a chloramphenicol acetyltransferase expression assay system, in both the presence and absence of constructs expressing the viral nonstructural protein, NS1. The ability of NS1 to transactivate the P38 promoter (up to 1,000-fold) depended entirely on the presence of intact GC and TATA box sequences. Disruption of the TAR element by either linker insertion mutations or an internal deletion did not inhibit transactivation of the P38 promoter. These results suggest that NS1 transactivates the P38 promoter indirectly by interacting with one or more components of the P38 core-transcription complex.

Minute virus of mice (MVM) is an autonomous parvovirus with two promoters, one at map unit 4 and the other at map unit 38 (4). The P4 promoter produces a transcript which encodes the nonstructural proteins NS1 and NS2. The NS1 protein transactivates the promoter at map unit 38, leading to a significant increase in the level of transcripts encoding the viral coat proteins VP1 and VP2 (10, 21). Neither the mechanisms by which NS1 transactivates the P38 promoter nor the sequences in the P38 promoter which are required for transactivation are known. Previous studies (17) on the related parvovirus H1 suggested that a 30-bp region of the H1 P38 promoter, positioned between −137 and −116 relative to the transcriptional start site, was essential for NS1 transactivation. A similarly positioned and homologous sequence to the putative transcriptional activator region, termed TAR, exists in the MVM P38 promoter. Li and Rhode (10) have also shown that conversion of a conserved lysine residue in the ATP binding site of H1 NS1 to a serine residue abolishes transactivation by NS1. While these results strongly indicate a role for the ATP binding site in NS1 activity, the mechanism of H1 NS1 transactivation is still unclear.

Previously, we defined the sequence elements in the MVM P38 promoter which were necessary for the basal level of transcription from the uninduced P38 promoter (6). In the absence of transactivation, the only functional elements detected in the promoter, both in vitro and in vivo, were a GC box and a TATA box. In this report, linker insertion and deletion mutations were used to refine our analysis of the sequence elements required for basal level transcription of P38 in vitro, as well as to define the sequence elements necessary for transactivation in vivo. Mutations in the TAR element had no effect on the ability of NS1 to transactivate the P38 promoter, whereas mutations in either the GC box or TATA box resulted in markedly lower levels of transactivation.

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MATERIALS AND METHODS

Construction of 5′-deletion mutations. The wild-type P38 construct, p38RH, is an *Eco*RI-*Hin*dIII fragment (nucleotides [nt] 1085 to 2650) subcloned into the Bluescript vector pBSKS+ (6). The deletion mutations were constructed by linearizing p38RH with *Bsm*I (nt 1790) and then digesting with *Bal* 31 nuclease. The digestion was stopped by the addition of an equal volume of EDTA, and the reaction products from various time points were analyzed on agarose gels. The appropriate-sized fragments were electroeluted from the gel, phenol-chloroform-isoamyl alcohol extracted, *Eco*RI digested (to remove upstream viral sequences), and purified on an agarose gel. The ends of the digested fragments were repaired by a filling-in reaction with the Klenow fragment of DNA polymerase I. Then, *Bgl*II linkers (GAA GATCTTC) were ligated to the ends of the fragments, and after digestion with *Bgl*II, the fragments were purified on low melting temperature agarose and recircularized by intramolecular self-ligation. The recovered plasmids were sequenced to verify the deletion endpoints.

Construction of 3′-deletion mutations. The 3′-deletion mutations were constructed in a modified pBSKS+ vector, pBSX, which had the single *Xho*I site in the polylinker removed by filling in the ends of the *Xho*I-cleaved plasmid with the Klenow fragment of DNA polymerase I and recircularizing. An *Eco*RI-*Hin*dIII fragment (nt 1085 to 2650)

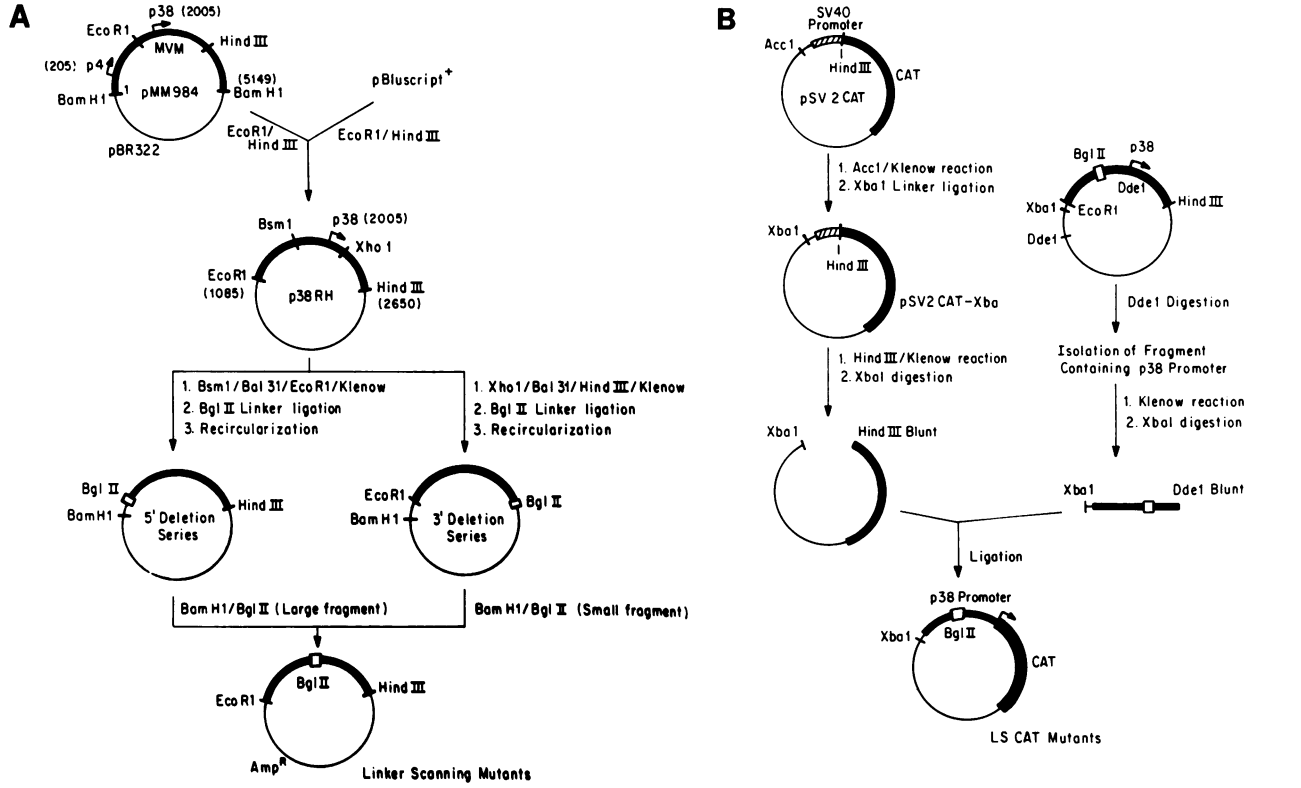

FIG. 1. (A) Construction of the linker scanning mutations in the P38 promoter of MVM(p) is diagrammed. (B) Construction of the pP38CAT linker insertion series is outlined. The *Xba*I-*Dde*I fragment containing the *Bgl*II linker substitution can be resolved from a similar-sized fragment derived from the vector by digestion with *Ssp*I. SV40, simian virus 40.

from the plasmid pMM984 was subcloned into pBSX to yield p38RHX. p38RHX was linearized with *Xho*I and treated with *Bal* 31 nuclease. The MVM sequences which were 3′ to the *Xho*I site were removed by digestion with *Hin*dIII. The plasmid DNAs were purified on agarose, and *Bgl*II linkers were added in a manner analogous to the 5′-deletion mutations.

Linker scanning mutations. Linker scanning mutations were constructed by ligating an *Apa*I-*Bgl*II fragment from a plasmid containing an appropriate 5′-deletion mutation into an *Apa*I-*Bgl*II-digested plasmid containing a 3′-deletion mutation (Fig. 1A). The LS pseudo clone was constructed by ligating together a (+4) 3′ deletion to a (+9) 5′ deletion, together resulting in a linker insertion mutation in which 5 bp of the wild-type sequence is duplicated.

Construction of LS CAT plasmids. The wild-type P38 promoter and linker scanning promoter mutants were cloned into a modified pSV2CAT vector, pSV2XCAT. pSV2XCAT was made by cleaving pSV2CAT at the *Acc*I site, filling in the ends with the Klenow fragment of DNA polymerase I, ligating *Xba*I linkers (CTCTAGAG) to the blunt ends, and recircularizing the plasmid (Fig. 1B). The promoter-containing sequences from pSV2XCAT were removed by digestion with *Hin*dIII and filling in with the Klenow fragment of DNA polymerase I, and then by digestion with *Xba*I. The P38 promoter fragment was obtained by digestion with *Dde*I and *Xba*I. The P38 promoter fragment was unidirectionally ligated into the promoterless pSV2XCAT vector to produce p38XCAT and pLS(1-15)CAT plasmids. The internal deletion mutations pID(−163/−72)CAT and pID(−163/−67)CAT were constructed by replacing the *Bam*HI-*Bgl*II fragments from plasmids pLS(−82/−72)CAT and pLS(−79/−67)CAT with the *Bam*HI-*Bgl*II fragment from pLS(−163/−154). The plasmid pKONS1/2 was derived from pKONeo (18) by replacing the *neo* gene with the MVM-coding sequence nt 225 to 3453.

In vitro transcription and primer extension analysis. Supercoiled plasmids containing either wild-type P38 sequences or linker scanning mutations were transcribed in vitro with A92L nuclear extracts as previously reported (2). Primer extension analysis of the in vitro transcription products used a 34-nt primer [nt 2123 to 2090 of the MVM(p) minus strand] (6). The sequencing ladder was generated by transcribing the plasmid p38-90 (which contains the P38 promoter truncated 90 nt upstream of the initiation of transcription) with T7 RNA polymerase and reverse transcribing the RNA products with avian myeloblastosis virus reverse transcriptase. The primer-extended samples were run on an 8% polyacrylamide-urea sequencing gel.

DNase I footprint analysis. Templates for DNase I footprinting were prepared by digesting plasmids bearing either the wild-type or linker insertion-mutated P38 promoter with *Nco*I and then *Bam*HI or *Xho*I and then *Bsm*I. The DNase I footprinting conditions have been previously described (16). Four femtomoles of probe and the indicated amounts of affinity-purified mSp1 were used.

Transfection assays. Plasmid DNAs were introduced into A92L cells by DEAE-dextran-mediated transfection (18). A92L fibroblasts were plated at a density of 1.5×10^6 cells per 100-mm dish in Dulbecco modified Eagle medium with

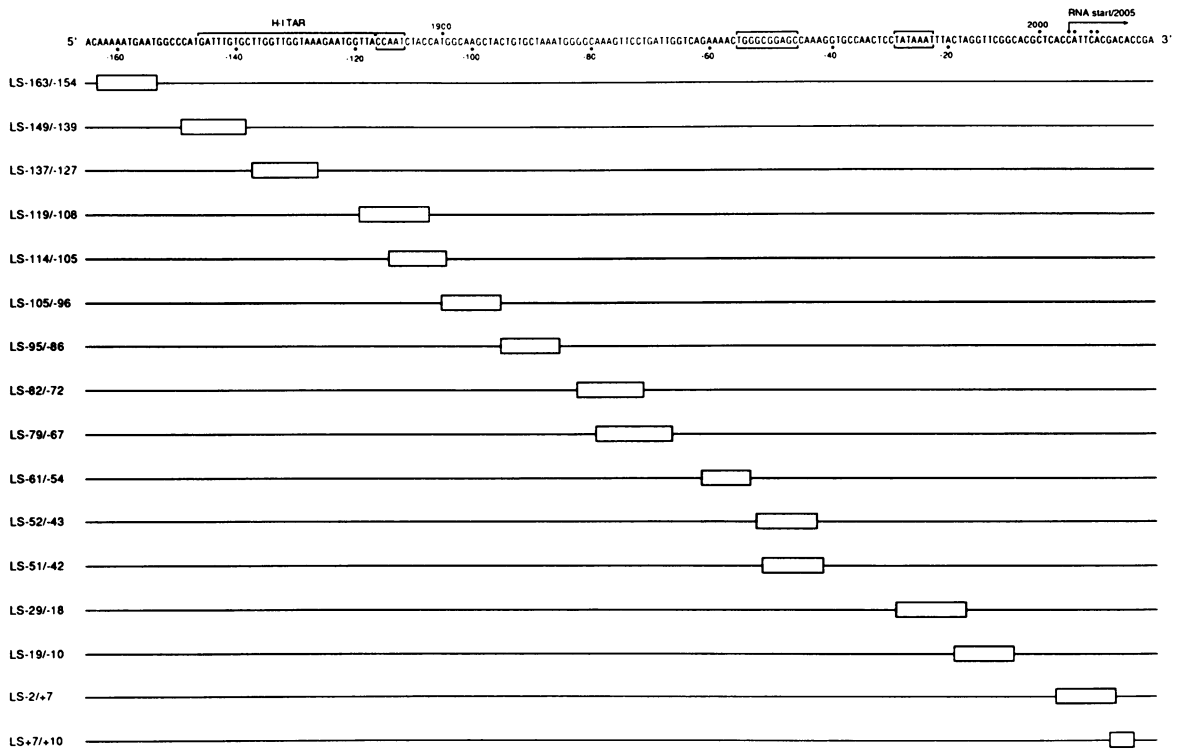

FIG. 2. Nucleotide sequence of the P38 promoter from MVM(p) is shown. The majority of transcripts initiate at nt 2005. The position of the homology to the H1 parvovirus TAR element is indicated with a bracket. Below the sequence, the nucleotide position of the linker scanning mutation relative to the start site of transcription is indicated on the left. The open boxes indicate the positions of the linker substitution.

10% fetal calf serum. At 24 h after plating, 4 μg of chloramphenicol acetyltransferase (CAT) vector ± 0.5 or 1 μg of pKONS1/2 were ethanol precipitated and resuspended in Tris-buffered saline. The cells were washed with phosphate-buffered saline (PBS), and 4 ml of fresh Dulbecco modified Eagle medium (10% fetal calf serum) was added to each plate. An 80-μl portion of warm (37°C) DEAE-dextran (10 mg/ml) was mixed with 40 μl of DNA, the DEAE-DNA mixture was added dropwise to a plate of cells, and the plate was gently rocked and returned to the incubator. After 4 h, the cells were washed twice with PBS and then shocked with 5 ml of 10% dimethyl sulfoxide for 1 min. After the plates were washed twice with PBS, 8 ml of medium was added to each plate. The cells were harvested 48 h after transfection, and CAT extracts were prepared and assayed (18). The reaction mixtures were extracted with ethyl acetate and spotted on silica thin-layer chromatography plates, and the plates were developed with 19:1 chloroform-methanol (vol/vol). After air drying, the plates were autoradiographed. Acetylated chloramphenicol was quantitated with a phosphor imager (Molecular Dynamics).

To provide the quantatative data in Fig. 7, we cotransfected the β-galactosidase expression vector pCH110X (4 μg) with the pKONS1/2 (1 μg) construct and the indicated P38CAT linker insertion constructs (4 μg). Cellular extracts were made as described above. Each extract was assayed by the method described by Gavin and Ward (6). The transfection efficiency was calculated from the specific activity of β-galactosidase present in each extract. Plasmid pKONS1/2 was a gift from D. Pintel. Plasmid pCHIV0 was a gift from P. Berg.

RESULTS

Construction and in vitro analysis of linker scanning mutations. We extended our analysis of the transcriptional regulation of the P38 promoter of MVM(p) by creating a family of *Bgl*II linker scanning mutations in the P38 promoter. The recognition sequence for the restriction enzyme *Bgl*II was chosen because it does not occur in the MVM(p) genome. Sixteen linker scanning mutations were created (Fig. 1A). Figure 2 shows the positions of the linker substitutions underneath the MVM sequences which were replaced.

The effect of the linker substitution mutations was assayed in an in vitro transcription assay with nuclear extracts from A92L fibroblasts (16). The RNA produced in these reactions was analyzed by primer extension (6). The primary RNA initiation site was observed at nt 2005 (Fig. 3, lane WT). Substituting the *Bgl*II linker sequence for sequences from −163 to −86 did not affect the level of transcription from the P38 promoter in vitro. The linker substitution mutations which replaced parts of the TAR sequence element (Fig. 3, lanes LS−149/−139, LS−137/−127, and LS−119/−108) had essentially wild-type levels of transcription. Inhibition of transcription was observed in the mutated promoter constructs in which either the GC box (LS−61/−54 and LS−52/−42) or the TATA box (LS−29/−19) sequence was replaced by the *Bgl*II linker sequence. Interestingly, when the sequences surrounding the site of the initiation of transcription were replaced (LS−2/+7), transcription in vitro was reduced at least 50% relative to wild type. An apparent increase in transcriptional activity was observed when the sequences downstream of the TATA box (LS−19/−10) were replaced.

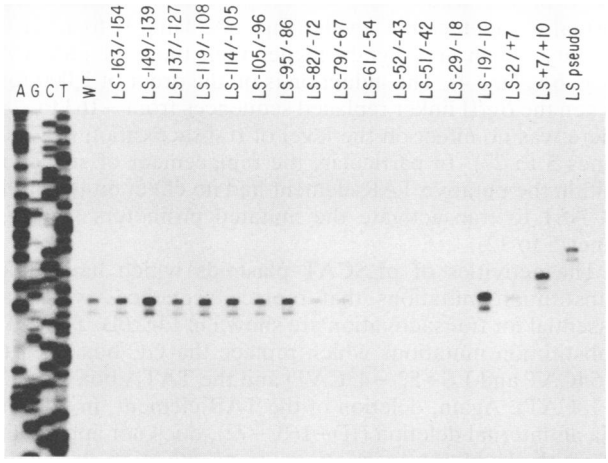

FIG. 3. Autoradiogram of the primer extension products from in vitro transcription assays, using the linker scanning P38 promoter mutants as templates. The primer extension reaction products were run on an 8% polyacrylamide-urea sequencing gel. There is a dominant transcript which initiates at nt 2005 and minor transcripts which initiate at nt 2006, 2009, and 2010. The template used in a particular in vitro transcription reaction is indicted above the lane. Lanes A, G, C, and T are dideoxy-sequencing ladders which were run on the gel to precisely identify the site of the initiation of transcription. WT, wild type.

The apparent change in start site in the primer-extended products from two of the mutated P38 templates (Fig. 3, lanes LS+7/+10 and LS pseudo) is due to a net insertion downstream of the start site of 6 nt in one and 15 nt in the other. Both templates showed wild-type levels of transcription.

The effects of the linker insertion mutations on the binding of Sp1 and other nuclear proteins to the P38 promoter was analyzed by DNase I footprinting in vitro. Figure 4 shows the results of a DNase I footprinting reaction in which affinity-purified murine Sp1 (mSp1) (16) was bound to end-labeled DNA fragments containing either the wild-type (WT) or mutated (LS 5′/3′) P38 promoter-positive strand which contains the G-rich sequence of the GC box. When *Bgl*II linkers replaced the GC box sequences (LS−61/−54 and LS−53/−43), mSp1 was unable to bind the mutated P38 promoter. Replacement of either the TATA sequences (LS−29/−18) or the sequences surrounding the site of the initiation of transcription (LS−2/+7) with a *Bgl*II linker had no effect on mSp1 binding. Similarly, a fragment containing the apparent up mutation (LS−19/−10) was bound by mSp1 in a manner similar to the wild-type promoter fragment. When a fragment containing the putative TAR sequence was incubated with crude nuclear extract and then treated with DNase I, only the GC box sequence was protected from

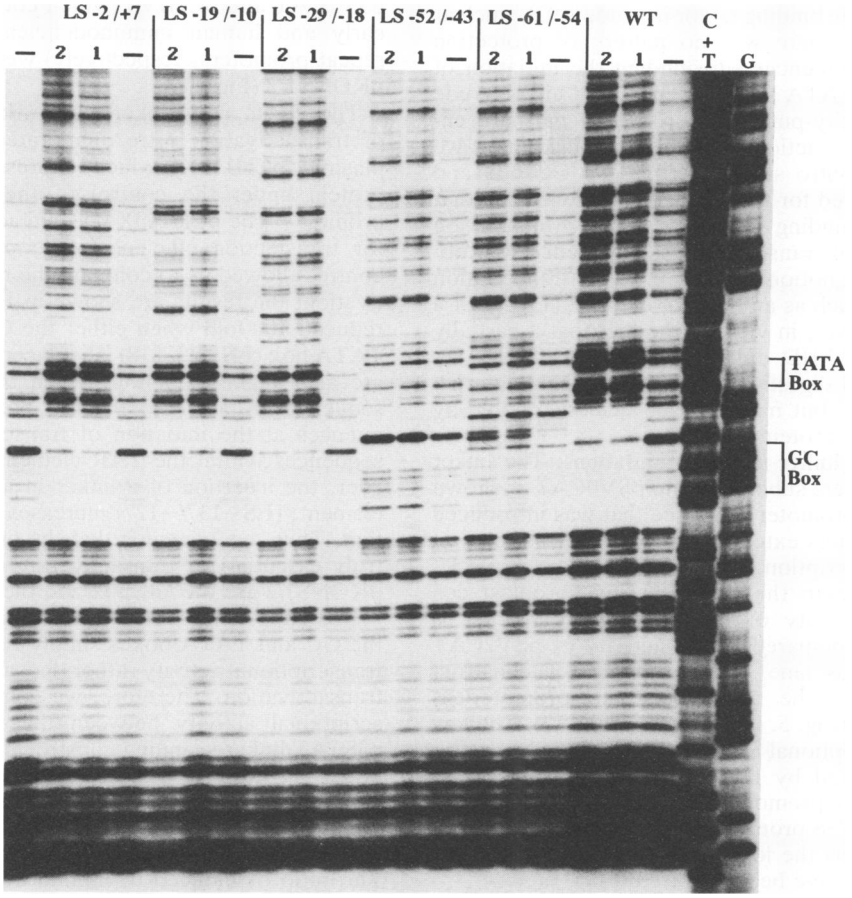

FIG. 4. DNase I footprinting of the P38 linker scanning mutant promoters with affinity-purified mSp1. The templates used are indicated above the lanes. The amount of mSp1 used was −, 0 μl; 1, 1 μl; and 2, 2 μl. Maxam and Gilbert C+T and G sequencing reactions were run as sequence markers. The GC box and TATA box sequence positions are indicated on the right side of the figure. Although the no protein lanes in some of the reactions are slightly overdigested, the DNase I cleavage sites in the middle of the GC box can still be seen.

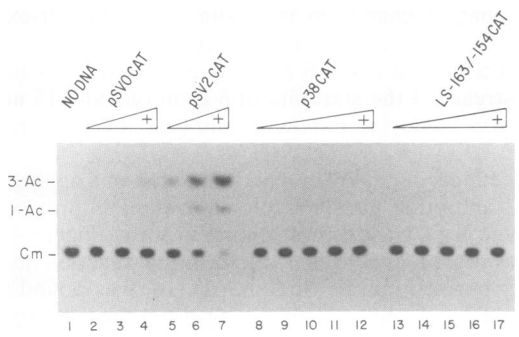

FIG. 5. Autoradiogram of a thin-layer chromatography plate showing the reaction products from CAT assays done with extracts made from A92L fibroblasts transfected with the indicated CAT constructs. The activity of the p38CAT and the LS−163/−154 CAT constructs was less than 0.1% of the pSV2CAT constructs. The amounts of DNA transfected are as follows: lane 1, 0 μg; lanes 2, 5, 8, and 13, 1 μg; lanes 3, 6, 9, and 14, 2 μg; lanes 4, 7, 10, and 15, 4 μg; lanes 11 and 16, 6 μg; and lanes 12 and 17, 8 μg. Cm, chloramphenicol; 1-Ac, 1-acetyl chloramphenicol; 3-Ac, 3-acetyl chloramphenicol.

digestion; no additional protection pattern was observed (1). Additionally, using the electrophoretic mobility shift assay with an end-labeled TAR element probe, we were unable to identify a TAR-specific binding factor in crude A92L nuclear extracts (5). Finally, there was no pattern of protection observed over the sequences surrounding the initiation of transcription or the TATA box sequence with either crude nuclear extract, affinity-purified mSp1, or a partially enriched murine TFIID fraction (15). The conclusion that we draw from these in vitro studies is that the GC box and TATA box are required for transcriptional activity and that the sequences surrounding the start site of transcription influences the level of transcription. No evidence was obtained to support the notion of other discrete transcription factor binding sites such as an initiator element (13, 20) or a TAR element. However, in view of the fact that a partially purified fraction of TFIID did not protect the TATA sequence from DNase I digestion, such protein-DNA interactions may still occur, but may only be demonstratable by using highly enriched protein fractions.

In vivo analysis of linker scanning mutations. The linker insertion mutations were subcloned into pSV0CAT as shown in Fig. 1B. The P38 promoter sequence that was introduced into the CAT constructs extended to 25 bp downstream of the start site of transcription.

When transfected into the A92L murine fibroblast cell line, the in vivo activity of the pP38CAT construct is undetectable when compared to the activity of pSV2CAT (Fig. 5, lane 7 versus lane 12). The pP38CAT construct activity is less than the activity of the promoterless pSV0CAT construct (Fig. 5, lane 4 versus lane 12). The low level of basal transcriptional activity from the P38 promoter in vivo was unaffected by the introduction of linker sequences outside of the promoter sequence elements (Fig. 5, lanes 13 to 17). The P38 promoter's weak in vivo transcriptional activity parallels the low levels of in vitro transcriptional activity which have been reported (5).

Figure 6 shows the induction of transcriptional activity of the p38CAT and pLSCAT constructs when they are cotransfected with pKONS1/2, a plasmid which expresses the MVM nonstructural genes NS1 and NS2. The transcriptional activity of the wild-type p38CAT plasmid is induced from virtually undetectable levels (Fig. 6A, lane 3) to levels of activity which are slightly higher than those of pSV2CAT (Fig. 6A, lane 4). The induction is on the order of 1,000-fold. When the *Bgl*II linker replaced sequences from −163 to −82, there was no effect on the level of transactivation (Fig. 6A, lanes 5 to 27). In particular, the replacement of sequences within the putative TAR element had no effect on the ability of NS1 to transactivate the mutated promoters (Fig. 6A, lanes 5 to 12).

The activities of pLSCAT plasmids which have linker substitution mutations that replace sequences which are essential for transactivation are shown in Fig. 6B. The linker substitution mutations which replace the GC box (LS−61/−54CAT and LS−52/−43CAT) and the TATA box (LS−29/−18CAT). Again, deletion of the TAR element, in this case via an internal deletion (ID−163/−72), does not appreciably effect the level of transcriptional transactivation. It is worth noting that the internal deletion which removes sequences up to −67 (ID−163/−67) and the linker scanning mutation LS−79/−67 both have decreased levels of activity even though the sequences which are replaced fall outside of the consensus sequence for the GC box. The activity of LS−2/+7 was decreased to about 30% of the wild-type activity. Additionally, pKONS1/2 appears to specifically transactivate only the P38 promoter and not other GC box-containing promoters. The levels of activity of pSV2CAT and pCHIV0 (which contain the simian virus 40 early and human immunodeficiency virus long terminal repeat promoters, respectively) were not transactivated by pKONS1/2 (Fig. 6C).

The effects of the linker substitution mutations on the level of transactivation were quantitated by cotransfecting the plasmid pCH110X, which expresses the β-galactosidase protein under the control of the simian virus 40 early promoter. The pCH110X plasmid acts as an internal control for transfection efficiency. The presence of the internal control allowed us to compare the relative levels of transactivation; the results are shown in Fig. 7. Transactivation is reduced 100-fold when either the GC box (LS−51/−42) or TATA box (LS−29/−18) is replaced by a *Bgl*II linker. There are also slight reductions when the *Bgl*II linker replaces sequences adjacent to the GC box (LS−61/−54), the sequences at the initiation of transcription (LS−2/+7), and sequences within the TAR element (LS−149/−139). However, the insertion of a linker in another part of the TAR element, (LS−137/−127) increases the level of transactivation. Thus, we conclude that the only sequences which are truly essential for transactivation of the P38 promoter by pKONS1/2 are the GC box and the TATA box.

It is important to exclude the possibility that mutations in the GC and TATA boxes simply reduced the basal level of transcriptional activity rather than altered the overall level of transactivation. Therefore, we compared the ratio of transcriptional activity between the induced and uninduced p38CAT linker scanning constructs (data not shown). If the underlying level of transactivation of the P38 promoter was constant despite the presence of linker scanning mutations, then the ratio of uninduced to induced would be the same for the various linker scanning mutants. This was not the case. The ratio of induced to uninduced levels of CAT activity from the p38CAT constructs gave an activity profile similar to that shown in Fig. 7; when the GC box or the TATA box is mutated, the ratio of induced to uninduced decreases, which is to say that the level of transactivation decreases.

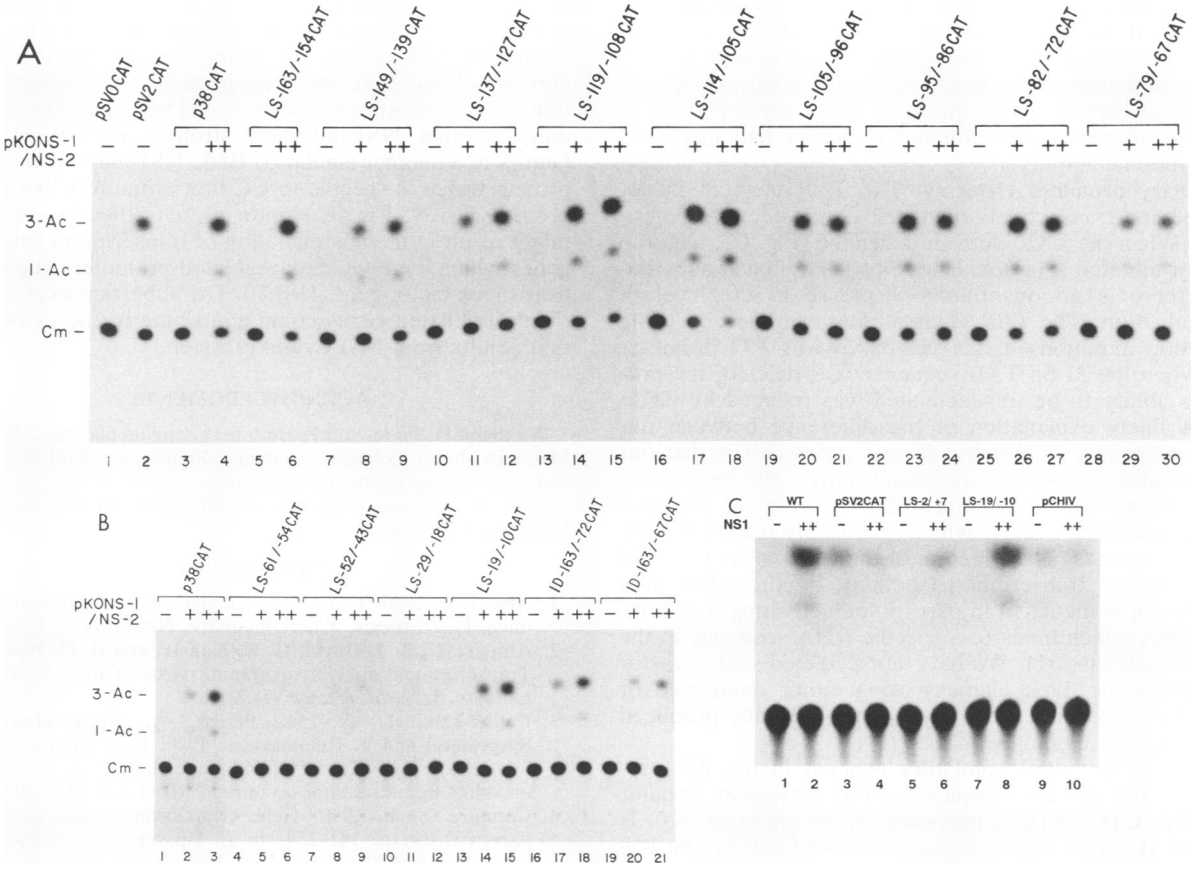

FIG. 6. Autoradiograms of thin-layer chromatography plates showing the reaction products from CAT activity assays done on extracts from A92L fibroblasts transfected with 4 μg of the indicated DNAs. The amount of cotransfected pKONS1/2 plasmid was either 0.5 μg (+) or 1 μg (++). WT, wild type. Cm, chloramphenicol; 1-Ac, 1-acetyl chloramphenicol; 3-Ac, 3-acetyl chloramphenicol.

DISCUSSION

P38 core promoter. The results presented here demonstrate that the GC box and the TATA box are the fundamental transcriptional regulatory elements in the P38 promoter. These results are in agreement with our previous work in which only 5′-deletion mutants were used (6). Transactivation of the P38 promoter by NS1 also requires both the GC and TATA boxes to be functional. Although the cotransfected pKONS1/2 expression vector produces both NS1 and NS2, it has been previously shown that NS1 is the protein which effects P38 transcriptional transactivation (21). Although a downstream element has been reported as participating in the in vitro transcriptional initiation from the P38 promoter (8), our results demonstrate that it is not required for transactivation of the P38 promoter.

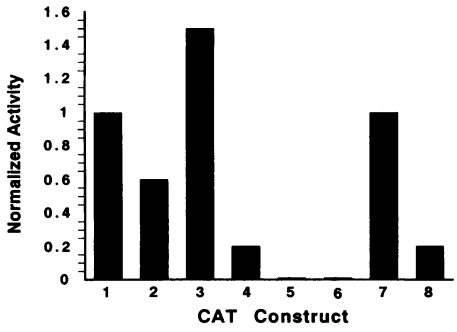

FIG. 7. Relative CAT activities from cells cotransfected with 4 μg of the indicated CAT construct and 1 μg of pKONS1/2. Cells were also cotransfected with 4 μg of pCH110X, expressing the *lacZ* gene. The LS CAT mutants that were transfected are: 1, LS−119/−108; 2, LS−114/−105; 3, LS−79/−67; 4, LS−61/−54; 5, −61/−54; 6, −29/−18; 7, −19/−10; 8, −2/+7.

The linker scanning mutations have an advantage over 5′-deletion mutations in that they do not disturb the spacing between the vector sequences and the promoter sequences. However, after analyzing the P4 and P38 promoters of MVM by using linker scanning mutations, it has become clear that there are sporadic enhancements and reductions in promoter activity when the *Bgl*II linker is placed at different points in the promoter. Interestingly, these changes in activity do not appear to correlate with the disruption of binding sites of sequence-specific DNA binding proteins.

In both the P4 promoter (LM−63/−54) (16) and the P38 promoter (LS−79/−67 and LS−82/−72), when a *Bgl*II linker is inserted adjacent to a GC box (Fig. 3, lanes LS−79/−67 and LS−82/−72), the transcriptional activity of the GC box is reduced even though there is no evidence that the wild-type sequences are actually contacted by the transcription factor Sp1 (15, 16). Since there is evidence that flanking sequences do influence Sp1's affinity for GC boxes (7, 12),

these results are not unusual; they simply underscore the influence of flanking sequences on the affinity of DNA binding proteins for their recognition sequences. The transcriptional enhancement in LS−119/−108 is an example of the opposite effect. When the *Bgl*II linker replaced part of the TAR sequence, the mutated promoter had increased transcriptional activity.

Auxiliary promoter elements. The TAR element is not required for transactivation in our cotransfection experiments. When the TAR element is deleted (Fig. 6C, lanes 16 to 18) or mutated (Fig. 6A, lanes 7 to 15) by linker substitutions, there is no quantitative decrease in the level of transactivation. The TAR element was identified by using 5′-deletion mutations in the H1 parvovirus P39 promoter (17). When the 31-bp TAR sequence was deleted, the promoter's ability to be transactivated was reduced by eightfold. A likely explanation of the difference between our results and those of Rhode and associates (17) is that our experimental protocols vary significantly. The case for a TAR element would be greatly strengthened by the presence of a DNA binding activity which specifically recognized the TAR sequence. Transactivation of the adenovirus E2A and E4 promoters' transcriptional activity by the adenovirus EIA protein is mediated in part by the activating transcription factor, which binds to a specific DNA sequence in the core promoter (9, 11). We have not detected such a factor recognizing the TAR element using either uninfected or infected cell extracts (5) or by adding bacterially produced NS1 into DNA binding reactions (14).

The other potential regulatory element in the P38 promoter is the initiator sequence. The canonical initiator sequence, CTCANTCT, proposed by Smale et al. (20) is matched at six of eight positions **CTCACCAT** by the P38 promoter sequence. Although the insertion of the *Bgl*II linker reduces the activity of the promoter fivefold in vivo (Fig. 7) and in vitro (Fig. 3), it remains to be seen whether or not the reduced activity is due to the mutation in the binding site of a sequence-specific initiation factor. The footprinting reactions which were conducted with crude A92L nuclear extract did not reveal specific protection of the initiator sequence as has been seen in the dihydrofolate reductase promoter (13). It could well be that the *Bgl*II linker insertion mutation is affecting other aspects of the transcription reaction and not the binding of an initiator protein.

Mechanisms of P38 transactivation. The data presented in this report suggest that the NS1 protein does not directly contact a specific DNA sequence in the P38 promoter but causes transactivation by modifying the activity of the core transcription complex, consisting of Sp1 and TFIID. There is no indication that NS1 requires sequences within the TAR element for transactivation of the P38 promoter. The target proteins which NS1 acts on are open to speculation. However, it does appear that NS1 acts catalytically, potentially as a kinase in transactivating P38 transcription (10). Additionally, our data demonstrate that the transactivation process increases the levels of transcription from the P38 promoter by up to 1,000-fold.

When NS1 is overexpressed in animal cells, it is cytotoxic (3). The cytotoxicity of NS1 contrasts with the activity of the adenovirus transactivating protein, E1A, which transforms cells when overexpressed. An E1A/E1B expression vector, pXC15, cannot transcriptionally transactivate the P38 promoter in cotransfection assays with p38CAT (15). This suggests that E1A and NS1 promote transcriptional transactivation in different ways, possibly by interacting with different sets of transcription factor proteins. It has recently been suggested that E1A acts as a transcriptional adaptor protein that mediates the interaction between the TATA binding protein and the upstream transcription factor Oct4 (19). Since the NS1 and E1A proteins are both transcriptional transactivating proteins, it will be interesting to determine whether NS1 interacts strongly with transcription factors in a manner similar to E1A. NS1 may be an adaptor protein which is specific to GC box promoters like the P38 promoter. If NS1 is an adaptor protein, then its expression might result in the misregulation of transcription from other genes which have GC box-regulated promoters such as the heat shock factor gene, HSF70. The abberrant expression of tightly regulated genes could contribute to the cytotoxicity that results from NS1 overexpression.

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