A cellular repressor regulates transcription initiation from the minute virus of mice P38 promoter

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ABSTRACT

We previously reported that the P38 promoter of minute virus of mice (MVM) is *trans* activated by the viral nonstructural protein, NS1, through an interaction with a downstream promoter element designated DPE. In this communication we report the identification of a distinct downstream promoter element which inhibits transcription from the P38 promoter *in vitro*, in the absence of the DPE. Removal of 34 bp from the region between +95 and +129 downstream from the P38 initiation start site relieved inhibition of transcription in whole-cell extract. Inhibition was also relieved by the addition, to the transcription reaction, of excess DNA fragments which span the putative inhibiting element. This indicated the involvement of a *trans*-acting factor, in inhibition of transcription from the P38. Gel retardation experiments demonstrated the specific binding of a cellular protein to the inhibitory element. This P38 inhibitory element shows spacing and orientation dependence as well as promoter specificity. The regulation of viral transcription by a cellular repressor may play an important role in obtaining a fine temporal order of viral gene expression during the course of infection.

INTRODUCTION

The expression of viral genes during infection of eukaryotic cells is temporally regulated and highly sensitive to cellular signals. In many of the documented cases, fine regulation is achieved through modular and complex interactions of viral and cellular elements. Cellular proteins may interact with viral DNA to regulate its transcription both positively and negatively. There are many examples of up regulation of viral transcription by cellular proteins: The cellular protein USF activates transcription from the adenovirus MLP through interaction with the upstream element (1). SP1 interacts with the 21 bp motif of the SV40 early promoter as well as many other viral promoters, and activates transcription (2). The murine AP1 homolog interacts with the polyomavirus enhancer and activates transcription from the viral early promoter (3). Much less is known, however, about the

interactions of cellular proteins with viral DNA which result in inhibition of transcription. One of the few examples is the interaction of the cellular protein, LBP-1 with the HIV-1 promoter upstream site (site II). This results in specific repression of transcription *in vitro* (4).

Minute virus of mice (MVM) is a linear single-stranded DNA parvovirus containing 5,149 nucleotides (nt). It is an autonomous virus but is dependent for replication upon cellular functions that are expressed during S phase (5, 6, 7). Transcription initiation catalyzed by the host RNA polymerase II has been shown to map to two promoters located at 4 (P4) and 38 (P38) map units (8). The two promoters have been more precisely mapped to nt 201 ± 5 and 2005 ± 4, respectively (9). Promoter P4 directs the transcription of two viral RNAs that are translated into the two nonstructural proteins, NS1 and NS2, while promoter P38 directs the transcription of the RNAs that are translated into the capsid proteins, VP1, VP2 and VP3 (6,10, 11). We have previously shown that the P38 promoter is controlled *in vitro* by a positive *cis*-acting element, which is located between nt 2287 and 2652 of the MVM genome and is defined Downstream Promoter Element (DPE). Furthermore, we suggested that the nonstructural protein NS1, is involved in the up regulation of transcription from the P38 promoter (12). Previous studies on the related H-1 parvovirus have implicated a role for a 30-bp upstream region in response to the NS1 (13), while other studies have defined the GC box and TATA element in the P38 promoter of MVM to be necessary and sufficient for *trans* activation by NS1 (14). In this communication, we report the identification of a distinct downstream promoter element which inhibits *in vitro* transcription from the P38 promoter. Its activity is apparent in the absence of the activating DPE. This element is contained within the 34 base pairs region located between nt 2095 and 2129 of the MVM genome. Its deletion from the transcribed template relieved inhibition. Moreover, when DNA containing this region was added *in trans* as a competitor, inhibition was relieved. The P38 promoter element is shown to have spacing and orientation dependence as well as promoter specificity. Finally, by gel retardation, a cellular protein is shown to bind this element specifically. We suggest that this interaction may account, at least in part, for the down regulation of transcription from the P38

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promoter early in MVM infection. We propose that later in infection, when the number of NS1 *trans*-activator molecules in the infected cell increases, this inhibition is overcome.

MATERIALS AND METHODS

Cells and extracts

HeLa cells were grown in suspension in Dulbecco's modified Eagle's medium supplemented with 5% calf serum. Whole-cell extracts (WCE) were prepared as described previously (15, 16).

Chemicals

Nucleoside triphosphates and creatine phosphate were purchased from Sigma Chemical Co. [α - 32 P] UTP (800Ci/mmol) was purchased from Rotem Industries Ltd. [α - 32 P] dATP, [γ - 32 P] ATP and dCTP (3000Ci/mmol) were purchased from Amersham International.

DNA templates used for *in vitro* transcription

Wild Type plasmid was constructed by insertion of a PstI–PstI MVM DNA fragment containing the P4 promoter (1 to 416 in the MVM numbering system) and an EcoRI–EcoRI MVM DNA fragment containing the P38 promoter (1084 to 3518 in the MVM numbering system) at the PstI site and EcoRI site in the polylinker of the pGEM 1 vector, respectively. 'Upstream' plasmid was constructed by deletion of an XhoI–BglII fragment (2070 to 3450) from wild type plasmid, filling in with the Klenow fragment and ligation. $\Delta(+58 - +95)$ was constructed by digestion of wild type plasmid with XhoI(2070) and mild digestion with Bal31 and re-ligation. $\Delta(+95 - +129)$ was constructed by first cloning SacI–PvuII fragment (containing the 1084–2840 fragment of MVM) between the SmaI and SacI sites in the polylinker of the pBluescript vector. This plasmid was subjected to site directed deletion mutagenesis by an oligonucleotide which flanks the deleted region: 5'-TCTGGGTTTCTGC AAGGC TCTAAA-3'. P4/P38 (+95 – +129) and P4/P38 (+129 – +95) were constructed from a plasmid in which the PstI–PstI MVM DNA (1 to 416, MVM numbering) was cloned at the PstI site in the polylinker of the pGEM 1 vector. The NcoI to HincII fragment containing MVM sequences 259 to 416 was deleted and the plasmid was filled in by Klenow fragment and ligated to a double stranded oligonucleotide spanning the region +95 to +129 of the P38 promoter: 5'-GAGCACACCAAATACTCC-TGTGCGGGCACTG-3'. Chimeric plasmids which have this insertion at either orientation were screened by DNA sequencing. The HIV I LTR/P38(+95 – +129) and HIV I LTR/P38(+129 – +95) was constructed from a plasmid which has the HIV I LTR sequences from 1 to 239 cloned at the HindIII site in the polylinker of the pBluescript vector by insertion of the same oligonucleotide as above at the HincII site in the polylinker and selection of chimeric plasmids which have this insertion at either orientation by DNA sequencing.

In vitro transcription with HeLa WCE

WCE (10 μ l) was incubated for 20 min at 30°C with DNA and 4mM creatine phosphate at a final volume of 15 μ l (preinitiation step). ATP, GTP, and CTP (final concentration 500 μ M), UTP (final concentration 10 μ M) and 20 μ Ci of [α - 32 P] UTP in 5 μ l were added for additional 45 min at 30°C. Reactions were stopped by the addition of 20mM EDTA, 20mM Tris–HCl (pH 7.9), 0.5% sodium dodecyl sulfate (SDS), 20 μ g proteinase K

and 5 μ g tRNA and incubated at 65°C for 25 min. RNA was collected by ethanol precipitation and centrifugation.

In reactions which included DNA competitors, agarose purified DNA fragments were added at the preinitiation step and reactions proceeded as described. Competitors used were either BstEII–NarI (MVM homologous), NarI–HindIII (MVM heterologous), or HinfI–HinfI fragments (pGEM 1 heterologous).

Gel retardation

Oligonucleotides spanning the region between +95 – +129, as described were annealed and end-labeled using T4 polynucleotide kinase and [γ - 32 P] ATP. A 100fmole (5000 cpm) were incubated with 20ng protein from WCE and 4 μ g poly [dI-dC]·poly [dI-dC] (Sigma Chemical Co.) in transcription buffer (20mM Tris–HCl [pH 7.9], 5mM MgCl₂, 0.1mM EDTA, 7.5% glycerol, 1mM dithiothreitol, 50mM KCl) for 15 min at 30°C after which in reactions including cold competitors, double stranded oligonucleotides were added for an additional 15 min at 30°C. Oligonucleotides used as heterologous competitors were: (HETI) 5'-ACTCCTGTTGCGGGC ACTG-3' and (HETII) 5'-CAGAAACCCAGAAC-3' both reflecting the immediate downstream region to the inhibitory element.

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Transcription: RNA was resuspended in 10 μ l running buffer (90% formamide, 1 $\times$ TBE) and analyzed on 4% or 6% polyacrylamide gels (bisacrylamide/acrylamide, 1:19) containing 7 M urea (Schwarz/Mann) and 1 $\times$ TBE (89mM Tris–borate [pH 8.3], 89mM boric acid, 2mM EDTA). Electrophoresis was carried out at a constant current of 20mA. Gel Retardation: DNA–protein binding reactions were loaded in 12% glycerol and analyzed on 6% polyacrylamide gels (bisacrylamide/acrylamide, 30:0.8) containing 1 $\times$ TG (25mM Tris–HCl, 250 mM glycine). Electrophoresis was carried out at a constant current of 50mA.

RESULTS

An inhibitory element located downstream from the P38 promoter

A DNA plasmid linearized 287 bp downstream to the P38 promoter was used as a template for *in vitro* transcription in HeLa whole-cell extract (WCE) (or nuclear extract, data not shown). We were unable to detect any run-off transcripts originating from this promoter, whereas transcription from the P4 promoter was clearly detected (Fig. 1, A, lane 2). Transcriptional attenuation or differential degradation were not detected (data not shown). Since we have shown that P38 is an active promoter in WCE reactions (12), the fact that transcription was not detectable using this DNA as template was surprising. The template DNA used in these experiments did not include the previously identified activating element, the DPE, which we have reported to interact with the viral protein NS1. This may account for a reduction in transcriptional activity. Alternatively, a distinct *cis*-acting element possibly down regulates this promoter. In order to distinguish between the two possibilities, we used a plasmid designated 'upstream' (see Fig. 1, B), as a template for transcription in WCE. In this plasmid, the upstream promoter region of P38 was kept intact, while the downstream region from +70 was deleted and replaced by irrelevant DNA from a plasmid. We reasoned, that if lack of transcription from a wild type

Figure 1. Activation of the wild type P38 promoter by removal of downstream sequences. Panel A: Transcription from a template in which the original downstream MVM sequences were replaced by non relevant DNA (0.5 μ g) and from a wild type promoter (0.5 μ g), Lanes 1 and 2, respectively. Location of run-off transcripts from P38 and P4 is indicated by arrows. M, 32 P end-labeled PBR322 DNA digested with MspI. Panel B: A Schematic representation of the plasmids used as templates for transcription, the location of the P4 and P38 promoters, the site of restriction digest and the expected run-off lengths in nucleotides.

template was simply due to the lack of the downstream activator, no transcription from the 'upstream' promoter will be detected, since it also lacks the DPE. On the other hand, if there is an inhibitory element in the region between +70 and +287, this inhibition will be relieved in the 'upstream' promoter, and transcriptional activity will be detected. Indeed, as can be seen in Fig. 1, A, lane 1, a transcript originating from the P38 promoter is synthesized from a template lacking MVM sequences downstream from +70. We have excluded the possibility that the fragment used to replace the native sequences had in itself an activating effect by checking other irrelevant fragments which all gave the same effect. This result has led us to conclude that the region between the vicinity of the start site and 287 bp from it, contains a *cis* element which is able to inhibit transcription. When this *cis* element is replaced by irrelevant sequences, transcription is regained even in the absence of the activating element. We therefore concluded the an inhibitory element is located downstream from the P38 promoter. Our results (data not shown) and the work of others (14) have revealed that in the absence of NS1, the activity of a P38CAT construct, lacking downstream sequences, when transfected into mouse fibroblast is completely undetectable. Due to this, and the fact that we were interested in further studying the effect of a downstream inhibitory element which acts in the absence of transactivation, we confined ourselves in the present study to the *in vitro* transcription system.

Figure 2. Specific and dose dependent relief of inhibition by DNA fragments spanning the putative inhibitory element. Panel A: Transcription from a wild type template (125 ng) digested with Nar I in the presence of 1 μ g (about 3.5 pmole) competing fragments: Lane 1, non specific, plasmid fragment. Lane 2, specific MVM fragment (-140 - +287, MVM numbering). Lane 3, non specific MVM fragment (+287 - +650). Panel B: Transcription as above in the presence of 0, 0.1 and 0.25 μ g (0, 0.35 and 0.9 pmole) specific MVM fragment. Location of run-off transcripts is indicated by arrows. M, size marker as in Fig. 1.

Inhibition of transcription from the P38 promoter is exerted through an interaction between a cellular protein/s and the downstream inhibitory element

Once we have demonstrated the existence of an inhibitory downstream *cis* element, we were interested to learn whether its activity is exerted through an interaction with a protein/s which recognizes it. To this end, we performed a transcription reaction in WCE using a wild type template in the presence of an excess of competitor DNA. We reasoned that if the inhibitory activity is indeed exerted through an interaction with a protein, the addition of excess DNA fragments spanning the region of the putative inhibitory element would relieve the inhibition due to titration out of all 'repressor proteins' from the extract. As seen in Fig. 2, A, lane 2, transcription from the P38 promoter is detectable upon the addition of a competitor DNA spanning the region between -114 and +287 relative to the P38 start site of transcription. This relief of transcription inhibition upon addition of competitor is specific, since as can be seen in Panel A, fragments from the region +287 - +650 of MVM (lane 3) or from a plasmid origin (lane 1) did not compete out the inhibitory effect resulting in a detectable P38 transcript. The ability of DNA fragments to compete away the repression is dose dependent as can be seen in Fig. 2, B, lanes 1, 2, and 3. S1 analysis confirmed that the transcript indeed originates from the P38 promoter (data not shown). The same results were obtained

Figure 3. Deletion of the region around +95 - +129 is necessary and sufficient for abolishing transcriptional inhibition. Panel A: Transcription in HeLa WCE was performed with various constructs as templates (0.5 μ g): Lane 1, Wild Type template digested with EcoRI and NarI, Lane 2, $\Delta(+58 - +95)$ template digested with EcoRI and NarI, Lanes 3 and 4, $\Delta(+95 - +129)$ template digested with StuI and NarI, respectively, Lane 5, wild type template digested with PstI and EcoRI. P4 transcripts and expected location of P38 transcripts is indicated by arrows. M, size marker. Panel B: Scheme of plasmids used as templates for transcription, the location of the P4 and P38 promoters and the expected run off lengths in nucleotides.

when a fragment spanning the region between +1 and +287 was used as the specific competitor (data not shown). We have not unequivocally determined whether the transcripts synthesized in this reaction used the full construct or the fragments as template. Nevertheless, the appearance of a transcript indicates that an excess of the region between +1 and +287 is able to relieve inhibition. However, we believe that the main portion of the appearing transcripts originate from the full construct, since such an excess of active promoter would otherwise cause inhibition of transcription due to calibration deviation. We concluded from this experiment that the addition of excess P38 promoter inhibitory element causes a functional depletion of a repressor protein from the WCE and this relieves transcription inhibition. This indicates that inhibition of transcription may arise from an interaction between a downstream inhibiting element and a cellular protein.

Determination of the minimal DNA region which is sufficient for inhibitory activity in transcription

To delimit the DNA region which is responsible for the inhibitory effect on transcription, our strategy was to delete small regions within the area spanning the inhibiting element (+1 - +287) separately. We assumed that in the absence of the sequences which account for inhibition, the promoter will be transcriptionally active. As can be seen in Fig. 3, lane 1, a wild type P38 promoter digested with Nar I, is not able to direct transcription (expected run off size is 287 nt) while the P4 promoter was active and directed the synthesis of the expected 253 nt long run off transcript. (See Fig. 3, B, a, for plasmid

Figure 4. Specific binding of a cellular protein from HeLa WCE to an oligonucleotide containing the inhibitory element. Gel retardation analysis of reactions containing 100 ng HeLa WCE proteins and 250 fmole probe. Lane 1, no competitor. Lanes 2, 3, 4 and 5 contain 2, 5, 10 and 20 fold molar excess homologous unlabeled oligonucleotide, respectively. Lanes 6, 7, 8 and 9 contain 2, 5, 10 and 20 fold molar excess heterologous unlabeled oligonucleotide, respectively. Lanes 10, 11, 12 and 13 contain 2, 5, 10 and 30 fold molar excess a second heterologous unlabeled oligonucleotide, respectively. Bottom: the sequence of the probe.

design). A plasmid bearing a deletion of the region +58 - +95 (see Fig. 3, B, b) was also unable to direct transcription from the P38 promoter (expected run off size is 250 nt), while the P4 promoter was active, as in the case of a wild type promoter (Fig. 3, A, lane 2). As can be seen in Fig. 3, A, lane 5, a plasmid in which the region between +129 and +287 was removed by linearizing the template with PstI at +129 was also unable to direct transcription (expected run off size is 129 nt) while the P4 promoter was active and directed the synthesis of the expected 217 nt long run off transcript (See Fig. 3, B, d, for plasmid design). These results indicated that the regions removed did not contain the inhibitory element. In contrast, the deletion of the region between +95 and +129 (see Fig. 3, B, c, for plasmid design) resulted in relief of transcriptional inhibition and the synthesis of a transcript originating from the P38 promoter as can be seen in Fig. 3, A, lanes, 3 and 4. This RNA was confirmed to be a P38 transcript by the size shift resulting from linearizing at the StuI and NarI sites. (Compare the size of the runoff transcript when the plasmid is digested with StuI, 343 bp downstream from the transcription start site, lane 3, and with NarI 253 bp downstream to the transcription initiation site, lane 4). We concluded from these experiments, that the region between +95 and +129 confers inhibition of transcription from the P38 promoter. Its deletion from the transcribed template is necessary and sufficient for the relief of inhibition.

The region between +95 and +129 is able to bind a cellular protein in a specific manner

We further wanted to study the mechanism by which the P38 downstream inhibitory element exerts its effect on transcription. To this end, we performed DNA retardation experiments to study any putative interaction of this element with a cellular protein/s.

Figure 5. The P38 inhibitory element is able to act on heterologous promoters in a promoter and orientation-dependent manner. Panel A: Transcription in HeLa WCE was performed with the following constructs as templates (0.5 μ g DNA): Lane 1, wild type P4 digested with EcoRI, lane 2, P4/P38 (+129 – +95) digested with BstNI, Lane 3, P4/P38 (+95 – +129) digested with BstNI, Lane 5, wild type HIV I LTR digested with HinfI, lane 6, HIV I LTR/P38 (+129 – +95) digested with HinfI, lane 6, HIV I LTR/P38 (+95 – +129) digested with HinfI. P4 and HIV transcripts are indicated by arrows. M, size marker. Panel B: Scheme of plasmids used as templates for transcription, the location of the P4 and HIV I promoters and the expected run-off lengths in nucleotides (nt).

A 34 bp oligonucleotide spanning the region between +95 and +129 was used as a probe. The probe was incubated with WCE and the resulting complex was resolved on native polyacrylamide gels. As can be seen in Fig. 4, lane 1, the inclusion of WCE in the binding reaction resulted in a retarded band. This band could be competed by an excess of cold homologous oligonucleotide (HOM) in a dose dependent manner, lanes 2–5, but not by an excess of two different heterologous oligonucleotides, lanes 6–9 (HET I) and 10–13 (HET II). We concluded from these experiments, that the region between +95 and +129 downstream from the P38 transcription start site is able to bind a cellular factor in a specific manner. Taken together with the observation that the deletion of this same region from the transcribed template resulted in a relief of transcriptional inhibition, and that excess of cold homologous fragments relieved inhibition in a dose dependent manner, these results suggest that the specific binding of a cellular protein to the region between +95 and +129 downstream to the P38 start site of transcription, causes an inhibition of transcription from this promoter. We shall refer to this cellular protein as a repressor.

The P38 downstream element is able to inhibit the P4, but not the HIV I LTR, in an orientation dependent manner

In order to study the promoter specificity of the P38 inhibitory element we have cloned it downstream to two heterologous

Figure 6. The activity of the P38 inhibitory element is distance dependent. Panel A: Transcription in HeLa WCE was performed with a plasmid containing an insert at position +70, digested with NarI or BglI, Lanes 1 and 2, respectively. Lane 3, wild type plasmid digested with NarI. M, ϕ X174 digested with HaeIII as size marker. Panel B: Scheme of plasmids used as templates for transcription, the location of the P4 and P38 promoters and the expected run-off lengths in nucleotides (nt).

promoters: The P4 promoter of MVM and the HIV I LTR. Thus creating chimeric promoters which bear the upstream regions of either the P4 or the HIV I LTR and the downstream region of the P38 promoter. The distance between the inhibitory element and the transcription start site was the same as in the original P38 template. The different chimeric plasmids were assayed for transcriptional activity in HeLa WCE. As can be seen in Fig. 5, A, transcription from the P4 promoter (run off size is 339 nt) was decreased when the inhibitory element was in the right orientation (Lane 3) compared to the activity of the original P4 promoter (run off size is 253 nt, lane 1). In contrast, the P4 promoter which contained the inhibitory element in the opposite orientation (lane 2) was as active as the original P4 promoter (see panel B for plasmid design). However, in the case of the HIV I LTR, the inhibitory element had no effect on the transcriptional activity of the promoter (run off size is 372 nt), in either orientation (Fig. 5, A, lanes 5 and 6) as compared to the original HIV I LTR (run off size is 338 nt, lane 4, see panel B for plasmid design). We concluded that the inhibitory element of the P38 promoter is able to repress transcription from some heterologous promoters but not from others and that its activity is orientation dependent.

Position effect of the inhibitory element relative to the start site of transcription

In order to find out whether the function of the inhibitory element is distance dependent, we inserted an irrelevant 700 bp DNA segment at nt +2070 between the start site and the inhibitory element. When a promoter carrying this insertion was used as template for transcription in WCE, it supported transcription (Fig.

6, panel A, lanes 1 and 2, run off sizes are 986 nt and 430 nt, respectively). The wild type P38 promoter supported no transcription, as observed before (lane 3, expected run off size is 287 nt). A similar plasmid with a different insert did not support transcription from the P38 promoter as well (data not shown). We concluded from these experiments that the activity of the inhibitory element residing downstream from the transcription start site is distance dependent. Its removal to a further downstream position results in its inactivation, therefore in a positive effect on transcription. However, we cannot exclude the possibility that by inserting a linker we have not only changed the distance between the start site and the inhibitory element, but also interfered with its configuration or its relative position on the DNA with respect to the upstream promoter region. This distance dependence experiment indicates that the location of the inhibitory element from the promoter region is critical for its effect on the promoter. We may speculate that the inhibitory element exerts its activity on transcription by somehow interacting with the transcription initiation complex which assembles at the promoter region.

DISCUSSION

In the present study, we report the identification of an inhibitory *cis* acting element that represses transcription from the P38 promoter of MVM and a cellular protein which interacts with it. A 34 bp DNA element residing downstream to the transcription start site of the P38 promoter of MVM is able to inhibit transcription *in vitro* in HeLa WCE. The deletion of a region immediately downstream to the P38 promoter abolishes inhibition and results in active transcription from this promoter. Systematic deletion of small segments from the downstream area of the P38 promoter, allowed us to identify the region between +95 and +129 as the inhibitory element. Furthermore, the involvement of a cellular protein in inhibition of transcription from the P38 promoter was indicated. The DNA element identified was shown by gel retardation to bind a cellular factor in a specific manner. Furthermore, this 34 bp DNA was able to confer inhibition on transcription from the P4 promoter of MVM but not from the HIV I promoter, when cloned downstream to these promoters. The element exerted its activity in an orientation dependent manner. This apparent promoter selectivity may result from a true specificity to an upstream or downstream sequence context. In this case, we may suggest that the transcriptional activity of a promoter reflects a concerted interaction between distinct promoter elements and *trans*-acting factors which bind to them. A certain combination will be inhibitory while a different one will have no effect. Alternatively, it may reflect a certain spatial stringency which was fortuitously kept in the P4 and not in the HIV I chimeric promoters.

Sequence analysis has not revealed any homology between the P38 inhibitory element and known transcription factor binding elements. This may indicate that the cellular protein inhibiting transcription from the P38 promoter of MVM is a yet unknown protein. Alternatively, a previously identified protein may interact with the P38 promoter downstream element as well as with other regulatory elements. We have previously identified a transcription activating *cis* element (DPE) residing further downstream from the P38 promoter start site (12). Thus, two distinct and different regulatory elements reside in tandem downstream from the P38 promoter. An inhibitory element which exerts its action through a cellular protein followed by an activating element which

interacts with a viral protein, NS1. The interplay between these regulatory effects may result in various levels of activity. It is noteworthy to mention that in conditions of activated transcription, when the DPE and *trans* activator NS1 are present, the effect of the inhibitory element is not pronounced (data not shown). It is possible that in the activated form, the binding site for the repressor is inaccessible. Alternatively, the activator and repressor proteins may compete for the same general transcription factors, in which case we must assume that the activator has a much higher affinity and/or is in large excess. We have recently purified a protein from HeLa nuclear extract that binds the inhibitory element (our unpublished results). We are currently investigating its nature and function in transcription.

There are a few possibilities to envision the mode of activity of a sequence specific DNA binding transcriptional repressor: a) It may compete with an activator by binding at or near its binding site. This was suggested in the case of transcriptional repression by the *Drosophila* segmentation gene product *Kruppel* (Kr) (17) and *Engrailed* (En) (18). b) A repressor may bind near the binding site of an activator and interact with it in a manner which inhibits activation (quenching), as been proposed for the *Drosophila*, *Even-skipped* (eve) and *en*, which inhibit transcription activation by *zen*-related *z2* through protein-protein interactions (19). This mode of repression is also thought to account for the inhibitory effect of the yeast mating type protein α 2 as exerted on the *a*-specific genes (20, 21). c) A repressor may interfere with the assembly of the general transcription factors, as suggested for SV40 T antigen (22), *eve* (23) as well as *c*- and *v-erbA* (24). d) A repressor may stabilize the binding of an inhibitory general transcription factor such as the negative general factors NC1 or NC2 (25, 26) or, e) A repressor may induce an unfavorable change of DNA conformation which may affect its accessibility to the transcriptional apparatus as suggested for the glucocorticoid receptor in transcription mediated by the CRE (27). Whatever may be the mode of repression, by which a cellular protein inhibits transcription from the viral promoter, it is intriguing to speculate about the advantage for the virus to conserve a sequence which represses transcription from its own promoter. The answer may lie in the temporal regulation of viral protein synthesis during infection. It has been established that, early in infection of synchronized A9 cell cultures, only the P4 promoter which directs the synthesis of the NS proteins is active (28). We, therefore, suggest that early in MVM infection, the P38 promoter is inhibited by the activity of the cellular repressor. Later in infection, the P4 promoter is down regulated by transcription attenuation (9, 29, 30) while NS1 which has reached a certain threshold concentration in the cell, activates transcription from the P38 promoter through an interaction with the DPE. Activation is suggested to abolish transcription inhibition by a mechanism which is yet unknown. Such putative interplay of activation and inhibition may equip the virus with a mechanism for the fine tuning of its gene expression during the course of infection which may prove essential for its efficient propagation. It allows the virus to concentrate on the replication of its genetic material during the early stages of infection while later in infection it enables it to produce the structural components towards the exit from the host cell. A simple activation model would not yield such a sensitive probe for the temporal shift in gene expression. The addition of a repressor to the picture ensures that a stoichiometrical balance is kept between the genetic material and its capsid which will be stringent as well as rapidly responsive to the environment.

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