JOURNAL OF VIROLOGY, Dec. 1994, p. 7974–7985
0022-538X/94/$04.00+0

Vol. 68, No. 12

Targeting of Promoters for *trans* Activation by a Carboxy-Terminal Domain of the NS-1 Protein of the Parvovirus Minute Virus of Mice

DANIEL LEGENDRE[1]* AND JEAN ROMMELAERE[2]

Laboratoire de Biophysique et de Radiobiologie, Département de Biologie Moléculaire, Université Libre de Bruxelles, B 1640 Rhode St Genèse, Belgium,[1] and Deutsches Krebsforschungszentrum, Angewandte Tumorvirologie, and Formation INSERM U375, D-69009, Heidelberg, Germany[2]

Received 22 June 1994/Accepted 31 August 1994

The NS-1 gene of the parvovirus minute virus of mice (MVM) (prototype strain, MVMp) was fused in phase with the sequence coding for the DNA-binding domain of the bacterial LexA repressor. The resulting chimeric protein, LexNS-1, was tested for its transcriptional activity by using various target promoters in which multiple LexA operator sequences had been introduced. Under these conditions, NS-1 was shown to stimulate gene expression driven by the modified long terminal repeat promoters (from the retroviruses mouse mammary tumor virus and Rous sarcoma virus) and P38 promoter (from MVMp), indicating that the NS-1 protein is a potent transcriptional activator. It is noteworthy that in the absence of LexA operator-mediated targeting, the genuine mouse mammary tumor virus and Rous sarcoma virus promoters were inhibited by NS-1. Together these data strongly suggest that NS-1 contains an activating region able to induce promoters with which this protein interacts but also to repress transcription from nonrecognized promoters by a squelching mechanism similar to that described for other activators. Deletion mutant analysis led to the identification of an NS-1 domain that exhibited an activating potential comparable to that of the whole polypeptide when fused to the DNA-binding region of LexA. This domain is localized in the carboxy-terminal part of NS-1 and corresponds to one of the two regions previously found to be responsible for toxicity. These results argue for the involvement of the regulatory functions of NS-1 in the cytopathic effect of this parvovirus product.

The NS-1 protein of the parvovirus minute virus of mice (MVM) (prototype strain, MVMp) is an 83-kDa nuclear phosphoprotein that plays essential roles in a productive infection (13). NS-1 is necessary for both the amplification of MVMp DNA and the regulation of viral gene expression. The multiple NS-1 functions and corresponding polypeptide domains involved in these processes have not been fully determined at present. Biochemical studies have shown that the NS-1 protein of MVMp and the analogous Rep 78 and Rep 68 products of adeno-associated viruses (a group of defective *Parvoviridae*) possess helicase and ATPase activities (34, 35, 62). Rep 78 and Rep 68 are able to bind to and cleave the adeno-associated virus DNA replication origin at a specific site (34, 35). Similarly, NS-1 contributes to the resolution of the terminal palindromic sequences of MVMp DNA replicative intermediates (12, 17, 42). These observations, together with the covalent binding of NS-1 to the 5′ termini of both duplex and progeny single-stranded DNAs (14, 15), are consistent with the DNA topoisomerase-like activity proposed for this protein (2). The mechanism(s) by which NS-1 modulates gene expression and the localization of the functional domain(s) concerned are more elusive to date. NS-1 was shown to enhance to a moderate extent (two- to fivefold) expression from its own promoter, P4, and to strongly activate (more than 100-fold) the P38 promoter, which drives the structural transcription unit (10, 19, 20, 32, 51). Experimental data argue for the involvement of NS-1 interaction with the terminal repeats and of DNA replication in the stimulation of promoter P4 (32). Multiple *cis*-acting sequences, including a *trans* activation-responsive (tar) upstream motif, a GC box, a TATA box, and a downstream element, were reported to be important for the activity and NS-1 induction of promoter P38, but the relative contributions of these sequences to *trans* activation by NS-1 remain debated (1, 22, 30, 31, 38, 52, 57). Mutations disrupting either of these sequences decreased but failed to abolish the responsiveness of the P38 promoter to NS-1 (30), suggesting that this protein may enhance the formation or stability of an active transcriptional complex by interacting with several cellular factors bound to the promoter.

In addition to its functions in the viral life cycle, NS-1 was also shown to inhibit the growth of transformed cells (6, 8, 39, 40) and to interfere negatively (21, 39, 52) or positively (60) with gene expression programmed by some heterologous promoters. Although the underlying molecular mechanisms are largely unknown, it has been proposed that the former effect may be, at least in part, a consequence of the latter. Indeed, a genetic analysis has shown that both the transcription-regulating and cytotoxic activities of NS-1 are confined to the amino- and carboxy-terminal portions of the protein and can be dissociated from its replicative function (39). This cosegregation raises the possibility that NS-1 cytopathogenicity may involve the disregulation of the expression of essential cellular genes. A delay of several days is indeed necessary for NS-1 to kill neoplastic cellular clones that have integrated the NS transcription unit under the control of a conditional promoter (8). Even though NS-1 may induce other molecular disturbances, in particular at the level of DNA replication (58), the above-mentioned observations suggest that the transcriptional function of this protein may be relevant not only to the life cycle but also to the biological effects of parvoviruses.

Some functional and structural features suggest that the

* Corresponding author. Mailing address: Laboratoire de Biophysique et de Radiobiologie, Département de Biologie Moléculaire, Université Libre de Bruxelles, 67 rue des Chevaux, B 1640 Rhode St Genèse, Belgium.

NS-1 protein has similarities to the acidic class of transcriptional activators. These proteins were shown to contain two distinct functional domains involved in promoter recognition and transcription activation, respectively (for a review, see reference 50). The former domain allows the protein to bind directly or indirectly to the promoter sequence and positions the latter domain so that it can interact with some components of the transcription machinery and promote transcription. The activating region of these transcriptional regulators is characteristically acidic. Interestingly, the amino- and carboxy-terminal portions of NS-1, which proved to be involved in the regulation of promoter expression by this protein (39, 52, 54, 55), are also acidic, as apparent from the charge distribution over the whole polypeptide from various parvoviruses (39). As a corollary to the two-domain structure of acidic regulators, the activating region can also interact with the target protein when both polypeptides are off the DNA. If the target protein is present in limiting amounts, this interaction will result in the downregulation of promoters lacking the activator recognition sequence. This squelching phenomenon was reported for several strong activators and was even shown to affect expression from recognition site-containing promoters, under conditions in which the activator is overproduced (4, 25, 33, 43, 45, 49, 59, 63). NS-1 exerts similar paradoxical effects on transcription, depending on the dosage of the NS-1-expressing plasmid. Indeed, promoter inhibition was found to increase with the dose of NS-1 effector plasmid transfected, while P38 was maximally *trans* activated by small amounts of the viral gene (52). Furthermore, the broad range of promoters whose activity is downregulated by NS-1 suggests that their repression results from a general mechanism such as the squelching of an essential factor(s). It should also be stated that the putative two-domain structure of NS-1 is supported by the existence of *trans* activation-defective NS-1 mutants that have a dominant negative phenotype (54).

In this study, we investigated the mechanism by which NS-1 exerts its regulatory effects on gene expression driven by the parvovirus P38 promoter and two heterologous promoters. To this end, the NS-1 transcription unit was fused with the coding region for the DNA-binding domain (amino acids 1 to 87) of the *Escherichia coli* LexA protein. This strategy allowed us to test the squelching model of promoter *trans* inhibition by NS-1. In particular, we determined whether a promoter that is normally inhibited by NS-1 becomes *trans* activated when it is supplemented with LexA operator sequences to make it a target for LexNS-1 fusion protein binding. That being the case, the above-mentioned system was further used to demonstrate (i) that *trans* activation by NS-1 can be assigned to a carboxy-terminal domain of the protein and (ii) that responsiveness of the P38 promoter to NS-1 is, at least in part, mediated by the tar sequence.

MATERIALS AND METHODS

Effector plasmids. The structures of the following effector plasmids are shown in Fig. 1A.

(i) pSVLexNSC1. The coding sequence for the DNA-binding domain of the LexA protein (amino acids 1 to 87) was isolated from plasmid pMG204 (SV-LexA [29]) as an *Eco*RI-*Asp*700 restriction fragment. This fragment was fused in phase with the coding sequence for the carboxy-terminal portion of NS1 (amino acids 547 to 672) by substituting it for an *Eco*RI-*Nco*I restriction fragment in a derivative of pMM984 (infectious molecular clone of MVMp [44]) that lacks the sequence between the *Nar*I sites. The *Nco*I site was filled in prior to ligation and was regenerated in the resulting plasmid, pULB3267. The sequence encoding the fusion protein was then recovered by *Eco*RI-*Nar*I digestion and transferred into the corresponding sites of pULB3269, which contains the *Eco*RI-*Bgl*II restriction fragment of pMM984 (nucleotides [nt] 1084 to 3450 of MVMp) in the polylinker of the expression vector pSG5 (Stratagene). An additional *Eco*RI restriction site was created by oligonucleotide site-directed mutagenesis 9 nt upstream from the start codon of LexA. Finally, the leader sequence from the *lexA* gene, which contains LexA operator sequences, was eliminated by deletion of the corresponding *Eco*RI fragment.

(ii) pSVLexNSC1fr. pSVLexNSC1fr was generated from pSVLexNSC1 by filling in the unique *Mlu*I site contained in the LexA-coding sequence. This created a frameshift mutation and introduced a premature stop codon that prevents the NS-1-coding sequence from being expressed in this construction.

(iii) pSVLexNS and pSVLexNSinv. The LexNS fusion was generated by inserting the missing portion of the NS-1 sequence in the unique *Nco*I site of pULB3267, as an *Nco*I-*Nco*I restriction fragment from pMM984 (nt 259 to 1897 of MVMp). The subsequent cloning steps were as described for pSVLexNSC1, except that the presence of an *Eco*RI site in the NS-1-coding sequence required (i) a partial *Eco*RI digestion to excise the LexNS-coding sequence before transferring it to pULB3269 and (ii) a complete *Eco*RI digestion followed by the purification and religation of the fragments to delete the leader sequence of the *lexA* gene. pSVLexNSinv resulted from the inversion of the orientation of the *Eco*RI fragment comprising the LexA- and amino-terminal NS-1-coding sequences.

(iv) pSVNS. The *Nco*I-*Bst*EII restriction fragment of pMM984 (nt 259 to 1884 of MVMp) was substituted for the *Eco*RI-*Bst*EII fragment of pSVLexNS to remove the LexA-encoding sequence. The *Nco*I and *Eco*RI sites were filled in prior to ligation.

(v) pSVLex3241, pSVLexNSN1, and pSVLexNSN2. Plasmids pSVLex3241 and pSVLexNSN1 were constructed by replacing the *Eco*RV-*Xho*I fragment of pSVLexNS with those of pULB3241 (39) and pMMBal02 (39), respectively. Plasmid pSVLexNSN2 was obtained in a similar way, by replacing the *Eco*RV-*Bst*EII fragment of pSVLexNS with that of pMMBal30 (39).

(vi) pSVLexNSN3. pSVLexNSN3 was constructed by deleting the *Bst*EII-*Xho*I fragment of pSVLexNS and religating filled-in ends.

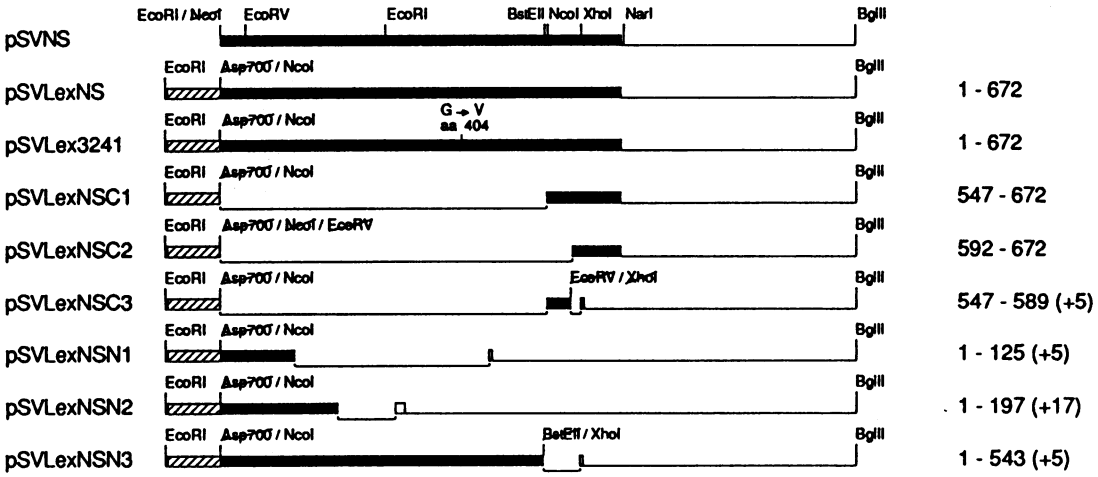
(vii) pSVLexNSC3 and pSVLexNSC2. pSVLexNSC3 was generated by deleting the *Eco*RV-*Xho*I fragment from a pSVLexNSC1 derivative (pSVLexNSC1M3) in which an *Eco*RV restriction site was introduced by oligonucleotide site-directed mutagenesis at nt 2030 of MVMp. The *Xho*I site was blunt ended before ligation. pSVLexNSC2 was constructed by substituting the 265-bp *Eco*RI-*Nco*I fragment of pSVLexNSC1, which contains the LexA-encoding sequence, for the *Eco*RI-*Eco*RV fragment of pSVLexNSC1M3. The *Nco*I site was blunt ended before ligation.

Reporter plasmids. The structures of the following reporter plasmids are shown in Fig. 1B.

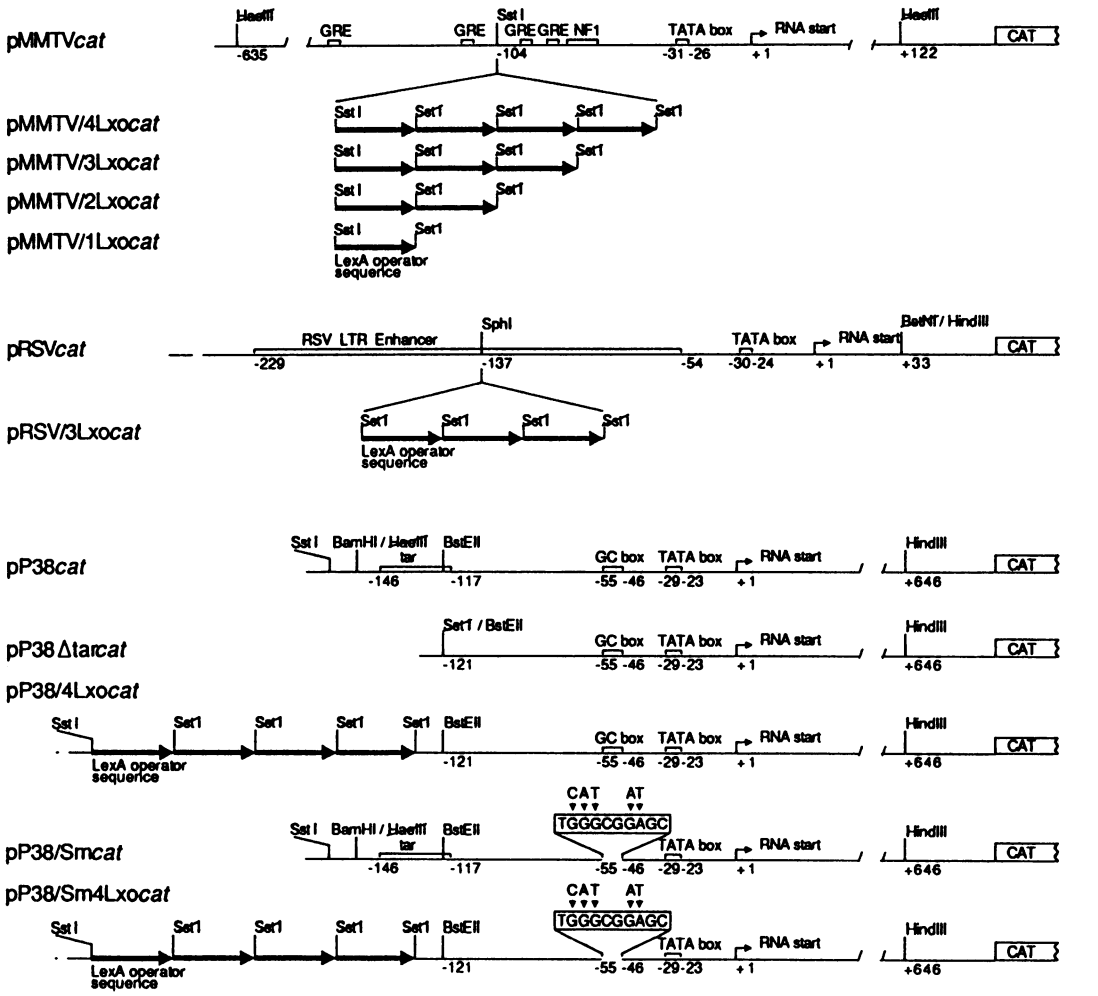
(i) pMMTV/1Lxo*cat*, pMMTV/2Lxo*cat*, pMMTV/3Lxo*cat*, pMMTV/4Lxo*cat*, and pRSV/3Lxo*cat*. Multiple LexA operator sequences were introduced into pMMTV*cat* (9) by incubating the *Sst*I-restricted plasmid with T4 DNA ligase and a high molecular excess (~300-fold) of the following double-stranded synthetic oligonucleotide:

```
                CGTACTGTATGTACATACAGTACGTCGACAGCT-3'
5'-TCGAGCATGACATACATGTATGTCATGCAGCTG
```

A. Effector plasmids



B. Reporter plasmids



This oligonucleotide contains the LexA operator sequence defined by Brent and Ptashne (7), flanked by cohesive *Sst*I ends. The number of inserted LexA operator sequences and their orientations were determined by restriction digestion and dideoxynucleotide sequencing. pRSV/3Lxo*cat* was constructed in a similar way by incubating *Sph*I-restricted and blunt-ended pRSV*cat* (26) with a molecular excess of the synthetic blunt-ended LexA operator sequence.

(ii) pP38Δtar*cat*. pP38Δtar*cat* was generated from pP38*cat* (8) by removing the *Sst*I-*Bst*EII fragment which contains the tar sequence defined by Rhode and Richard (52). The *Sst*I and *Bst*EII sites were filled in prior to ligation.

(iii) pP38/4Lxo*cat*. The mouse mammary tumor virus (MMTV) promoter from pMMTV/4Lxo*cat* was cloned in pSG5 as a 0.86-kb *Hin*dIII-*Bst*EII restriction fragment and modified by oligonucleotide site-directed mutagenesis to create a *Bst*EII site 12 nt downstream from the *Sst*I site. The four LexA operators were rescued as a 149-bp *Sst*I-*Bst*EII restriction fragment and cloned into the corresponding sites of pP38*cat* as a substitute for the tar sequence.

(iv) pP38/Sm*cat* and pP38/Sm4Lxo*cat*. The P38 promoter Sp1 recognition site (TGGGCGGAGC) from pSVNS was modified by oligonucleotide site-directed mutagenesis to TCATCGATGC and transferred as part of a *Bst*EII-*Hin*dIII fragment into the corresponding sites of pP38*cat*, to give pP38Sm*cat*. pP38/Sm4Lxo*cat* was obtained by substituting the four LexA operators from pMMTV/4Lxo*cat* for the tar sequence of pP38Sm*cat*, as described above.

Oligonucleotide site-directed mutagenesis. The cloning and expression vector pSG5 used in this study contains the replication origin of the f1 filamentous phage. This allows single-stranded DNA (ssDNA) to be rescued upon coinfection with a helper phage for use in subsequent mutagenesis. An internal deletion in the packaging signal of the helper phage (R408 [Stratagene]) ensures that plasmid ssDNA is packaged and exported in preference to the phage genome. Briefly, exponentially growing cultures of TG1 bacteria transformed by the above-mentioned pSG5 derivatives were inoculated with helper phages at a multiplicity of infection of about 100 particles per cell. After overnight incubation at 37°C with vigorous agitation, phages were recovered from the supernatant by NaCl-polyethylene glycol precipitation, and ssDNA was subsequently purified by phenol-chloroform extraction and ethanol precipitation. Oligonucleotide site-directed mutagenesis was performed with ssDNA, using the Amersham kit (version 2.1).

Cells. The simian virus 40-transformed human cell line NBE (newborn kidney cells) (53) was grown in minimal essential medium supplemented with 5% fetal calf serum.

DNA transfection and transient expression assay. NBE cultures (1.5×10^5 cells per 6-cm-diameter dish) were transfected with 1 μg of reporter plasmid and various amounts of effector plasmid by the Ca-phosphate precipitation method, as previously described (6). Samples were collected 48 h after transfection and assayed for chloramphenicol acetyltransferase activities as described by Gorman et al. (27). Chloramphenicol acetyltransferase activities were quantified by scintillation counting.

RESULTS

A chimeric gene was constructed in which the sequences coding for NS-1 and for the DNA-binding domain of the bacterial LexA repressor were fused. The LexA component confers to the fusion protein the capacity for recognizing a specific DNA sequence, the LexA operator, and hence for interacting with promoters in which this sequence has been introduced.

Functional state of the LexNS-1 fusion protein. To ascertain that the fusion of NS-1 with the DNA-binding domain of LexA did not alter the regulatory properties of the viral protein, NS-1 and LexNS-1 were compared for their effects on the activity of MVMp and heterologous promoters. The two proteins were expressed from the simian virus 40 early promoter, which proved not to be very sensitive to NS-1 (6), by using plasmids pSVNS and pSVLexNS, respectively. Western blot (immunoblot) analysis showed that cells transfected with these plasmids accumulated similar amounts of NS-1 and LexNS-1 polypeptides, indicating that the fusion did not affect protein production or stability (data not shown); also, the amount of NS-1 or LexNS-1 produced under these conditions was similar to that directed by the genuine P4 promoter after transfection with plasmid pMM984. The transcriptional functions of the LexNS-1 fusion protein were first tested by means of two target promoters known for their differential responsiveness to NS-1: the internal P38 promoter of MVMp and the long terminal repeat (LTR) of Rous sarcoma virus (RSV) (39, 52). The activities of these promoters in the presence of NS-1 or LexNS-1 were determined by cotransfecting the reporter plasmid pP38*cat* or pRSV*cat* with either pSVNS or pSV-LexNS. Plasmid pSVLexNSinv, which is deficient in NS production because of the inversion of the sequence encoding the 362 N-terminal amino acids of the fusion protein, was used as a negative control to assess the possible NS-independent interference of effector plasmid DNA. As observed previously with the effector plasmid pMM984 (39), gene expression driven by the P38 promoter was stimulated over 100-fold by NS-1, whereas expression programmed by the RSV LTR was

FIG. 1. Structures of effector and reporter plasmids. (A) Effector plasmids. The NS-1 and LexA–NS-1 fusion genes were inserted between the *Eco*RI and *Bgl*II cloning sites of the pSG5 expression vector, which contains the early promoter as well as splice and polyadenylation signals of simian virus 40. Sequences coding for the DNA-binding domain of LexA, part or all of the NS-1 protein, and out-of phase amino acids (aa) are represented by hatched, filled, and open boxes, respectively. Deletions in the NS-1-encoding sequence are indicated by underlying lines. The NS-1 amino acid stretches present in the various fusion proteins are given on the right, with the number of additional C-terminal frameshifted residues in parentheses. In pSVLex3241, mutations were introduced into the NS-1-coding sequence at nt 1471 and 1472, changing codon 404 from glycine (GGC) to valine (GTG). (B) Reporter plasmids. Plasmid pMMTV*cat* is a pUC8 derivative containing the transcriptional start site and upstream regulatory elements from the MMTV LTR (−635 to +122) fused to the *cat* gene, as well as splice and polyadenylation signals from pSV2*cat*. The glucocorticoid-responsive elements (GRE) and the binding site for nuclear factor 1 (NF1) are indicated. In pMMTV/Lxo*cat* derivatives, one to four LexA operator sequences were introduced 104 nt upstream from the transcriptional start site. Plasmid pRSV*cat* contains the U3-R region (−234 to +21) from the RSV LTR as a substitute for the simian virus 40 early promoter in plasmid pSV2*cat*. In the pRSV/3Lxo*cat* derivative, three LexA operator sequences were introduced at nt −137 from the transcriptional start site. Plasmid pP38*cat* contains the *cat* gene between the P38 promoter (−151 to +646) and polyadenylation signal of MVMp. The pP38Δtar*cat* and pP38/Sm*cat* derivatives were generated by deleting the tar sequence and mutating the GC box, respectively. In plasmids pP38/4Lxo*cat* and pP38/Sm4Lxo*cat*, four LexA operators were substituted for the tar sequence of plasmids pP38*cat* and pP38/Sm*cat*, respectively. CAT, chloramphenicol acetyltransferase.

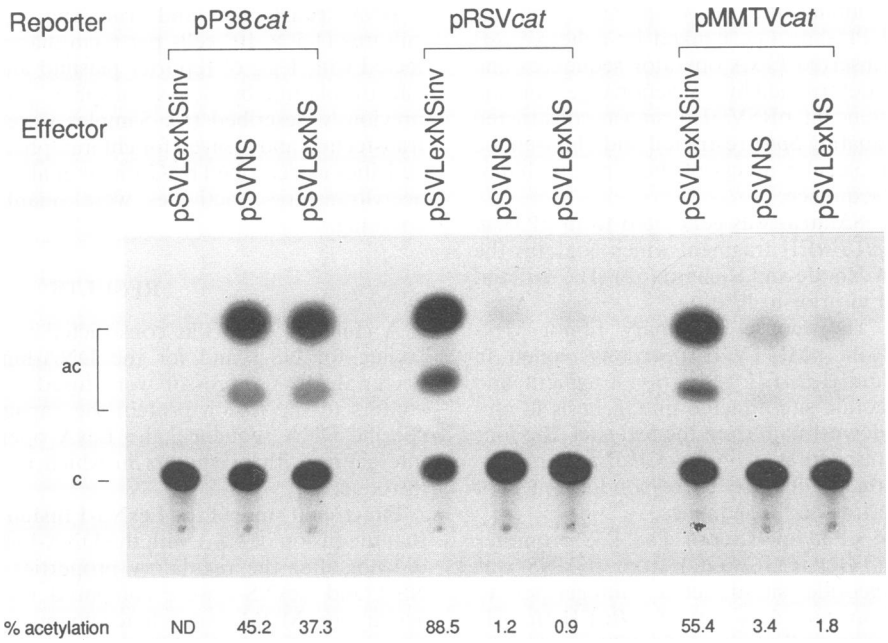


FIG. 2. Effects of the NS-1 and LexNS-1 proteins on reporter gene expression driven by parvovirus (P38) and heterologous (RSV and MMTV LTR) promoters. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of the indicated reporter plasmid and either 5 μg of pSVLexNS or an equimolar quantity of the other effector plasmids. After removal of the Ca-phosphate precipitate, cells transfected with the pMMTV*cat* reporter were incubated for 48 h in the presence of 10^{-5} M dexamethasone. Two- to 10-fold-larger amounts of cellular extract were used to measure *cat* expression driven by the RSV and MMTV LTRs, respectively, compared with promoter P38. The percentages of acetylated chloramphenicol are given at the bottom. c, ^{14}C-labeled chloramphenicol; ac, acetylated derivatives; ND, not detectable.

reduced to less than 10% of its normal level (Fig. 2). The effects of the LexNS-1 polypeptide on these two promoters were both qualitatively and quantitatively similar to those of wild-type NS-1 (Fig. 2), indicating that the transcriptional function of NS-1 was retained by the fusion protein. This property was further checked with another heterologous promoter, the LTR of MMTV. The basal activity of this promoter is barely detectable in NBE cells but is much enhanced in the presence of glucocorticoids. The pSVNS and pSVLexNS effector plasmids were tested for their abilities to interfere with the expression of pMMTV*cat* in cotransfected cells incubated with 10^{-5} M dexamethasone. Like the RSV promoter, the MMTV LTR was found to be inhibited by more than 90% in the presence of NS-1, whether or not the protein was fused with LexA (Fig. 2). This confirmed that the LexNS-1 protein is fully active as far as its transcriptional function is concerned.

Transcriptional activation by bound LexNS-1. The question arises as to whether a promoter that is *trans* activated by NS-1 can be distinguished from *trans*-inhibited ones by its ability to associate with the protein, i.e., whether *trans* activation by NS-1 requires the viral product to directly or indirectly interact with the promoter or with upstream or downstream sequences. The LexNS-1 system allowed us to address this question by determining if a promoter that is normally repressed by NS-1 becomes activated once it is supplemented with LexA operators to which the fusion protein binds through its LexA component.

The MMTV LTR was first used to this end. LexA operators were inserted into a unique *Sst*I site of the pMMTV*cat* reporter plasmid, at position −104 from the transcription start site (Fig. 1B). Four derivatives of pMMTV*cat* were isolated, in which one to four LexA operators were present in a head-to-tail orientation. In the absence of functional NS-1 (pSVLexN Sinv), the activities of these modified promoters were enhanced by dexamethasone, yet the extent of this stimulation was less than that with the wild-type MMTV LTR (Fig. 2) and decreased as a function of the number of LexA operator inserts (Fig. 3). This is probably due to the fact that the introduction of LexA operators at the *Sst*I site moves upstream glucocorticoid-responsive elements away from the proximal promoter region. The modified promoters were tested for their *trans* regulation by LexNS-1. In contrast with the wild-type promoter (Fig. 2), the derivatives containing two to four LexA operators were *trans* activated by LexNS-1 whether in the presence or absence of dexamethasone (Fig. 3). The stimulation level increased with the number of LexA operators. It is noteworthy that LexNS-1 failed to activate the promoter harboring a single LexA operator (data not shown), which may tentatively be attributed to the fact that the fusion polypeptide lacks the C-terminal LexA domain involved in stabilizing the DNA-protein complex (41). As shown in Fig. 4, the *trans*-activating effect of LexNS-1 on pMMTV/4Lxo*cat* required both the LexA DNA-binding and NS regulating components of the fusion protein (pSVLexNS), since it was not exhibited by wild-type NS-1 (pSVNS) and was abolished by a mutation affecting the NS-1 constituent of the chimeric polypeptide (pSVLexNSC1fr). It should also be stated that LexNS-1 was a potent *trans* activator of the promoter supplemented with four LexA operators, inducing it to achieve—in the absence of dexamethasone—a level of expression of the same order of magnitude as that of the glucocorticoid-stimulated wild-type promoter (Fig. 2 and 3). Furthermore, LexNS-1 proved to cooperate with dexamethasone in the activation of the modified MMTV LTR (Fig. 3), as previously reported for the yeast transcription factor Gal4 under comparable conditions (37).

In order to confirm these results in another system, similar

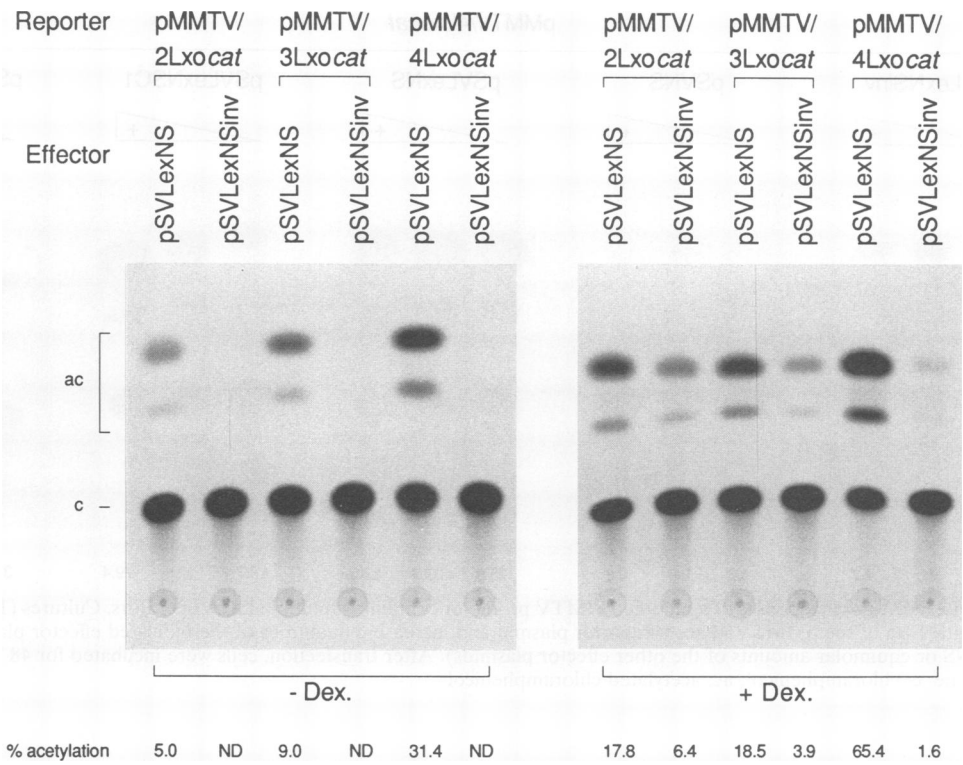


FIG. 3. *trans*-activating effect of the LexNS-1 protein on modified MMTV promoters containing multiple LexA operators. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of the indicated reporter plasmid and 50 ng of pSVLexNS or pSVLexNSinv effector. The Ca-phosphate precipitate was removed 4 h after transfection, and cells were further incubated for 48 h in the presence or absence of 10^{-5} M dexamethasone (Dex.). c, chloramphenicol; ac, acetylated chloramphenicol; ND, not detectable.

experiments were performed with the RSV promoter, whose inhibition by NS-1 is well documented (Fig. 2) (39, 52). Three LexA operators were introduced into the RSV LTR at a distance from the transcription initiation site similar to that for the case of the MMTV LTR (Fig. 1B). The promoter modified in this way, RSV/3Lxo, had a weaker basal activity than the wild-type RSV LTR, probably as a result of the insertion of LexA operators into the enhancer region (Fig. 2 and 5). In contrast with the RSV LTR (Fig. 2), RSV/3Lxo was found to be stimulated by the LexNS-1 protein (Fig. 5) to an extent (four- to fivefold) similar to that for MMTV/3Lxo (Fig. 3).

Together, these data show that the MMTV and RSV promoters, which are normally inhibited by LexNS-1, turn out to be *trans* activated once they are engineered so as to bind the fusion protein. This suggests that NS-1 possesses a transcription-activating function which cannot be exerted at a distance but requires the protein to be targeted on DNA control elements. The transcription factor(s) with which NS-1 presumably interacts is unknown but may be common, given the NS-1 responsiveness of the unrelated parvovirus (P38) and modified retroviral (LTR) promoters.

Squelching properties of free NS-1 and LexNS-1. In the absence of added LexA operators, the LTRs of MMTV and RSV not only failed to be *trans* activated by LexNS-1 but instead were inhibited by the fusion protein (Fig. 2). Likewise, the LexA-free NS-1 polypeptide was unable to stimulate and actually repressed the LexA operator-supplemented LTR promoters (Fig. 4 and 5). Consistently, this repression was also observed when both elements of the LexA system were missing, i.e., for unmodified LTRs in the presence of wild-type NS-1 (Fig. 2). Thus, the *trans* inhibition of LTRs by NS-1 proved to be conditional on the absence of a responsive element (Lxo) in the target promoter and/or of a corresponding DNA-binding domain (Lex) in the effector protein. This strongly suggests that the inhibitory function may be carried out by unbound NS-1 protein through the sequestration of an essential transcription factor(s), i.e., through a so-called squelching mechanism.

Squelching characteristically increases with the concentration of free activator, which, when present in excess, can even affect expression from promoters possessing the corresponding recognition motif. The NS-1 dose responses of promoter *trans* regulation were in agreement with these properties. First, the inhibitory effect of NS-1 on MMTV/4Lxo (Fig. 4) and RSV/3Lxo (Fig. 5) was most pronounced at the highest pSVNS plasmid concentration tested. Second, the *trans* activation of MMTV/4Lxo (Fig. 4) and RSV/3Lxo (Fig. 5) by LexNS-1 could be partly or totally masked by increasing the dose of effector plasmid. Also, the uptake or expression of pRSV/3Lxo*cat* was slightly increased in the presence of an excess amount of the pSVLexNSinv plasmid used as a negative control (Fig. 5). This stimulation was distinguishable from the LexNS inducing activity by its lesser extent and its dependence on a high effector plasmid concentration. The reason for this modulation is unclear but could not be attributed to the insert of pSVLexNSinv, because the parental pSG5 plasmid had a similar effect when present in excess (data not shown).

In conclusion, the respective conditions under which NS-1 (or LexNS-1) exerts positive and negative regulatory activities argue that this polypeptide possesses an activating domain which can stimulate or repress a responsive promoter, depending on whether a DNA-protein complex is formed.

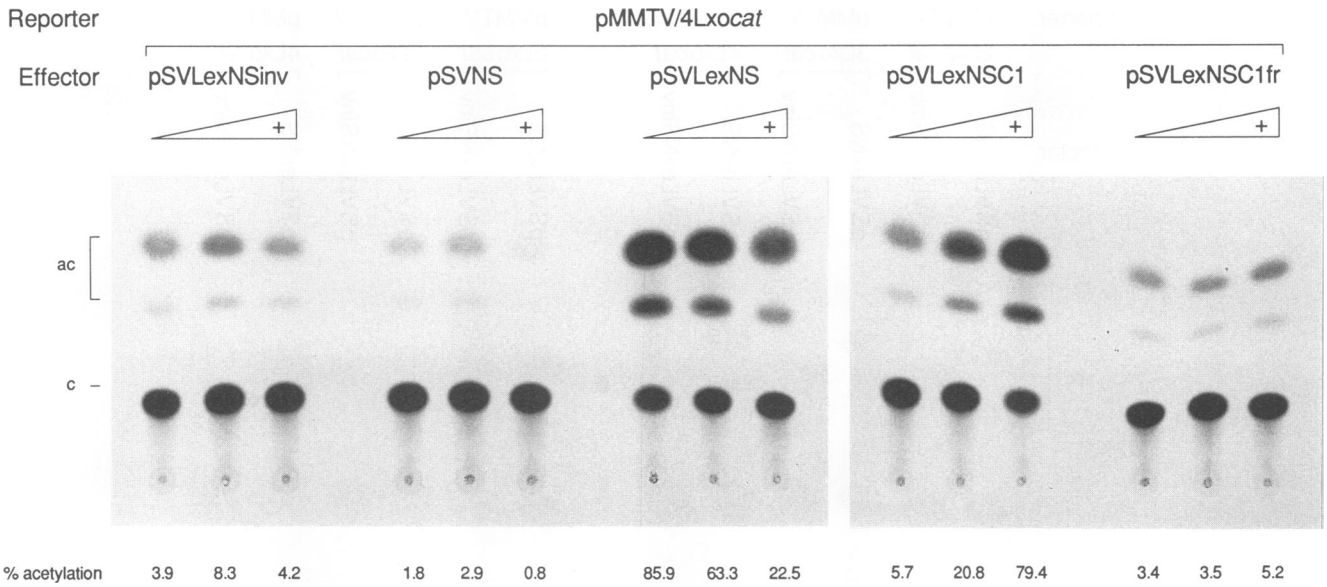


FIG. 4. NS-1 and LexNS-1 dose responses of a modified MMTV promoter containing multiple LexA operators. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of the pMMTV/4Lxo*cat* reporter plasmid and increasing quantities of the indicated effector plasmids (50, 500, and 5,000 ng of pSVLexNS or equimolar amounts of the other effector plasmids). After transfection, cells were incubated for 48 h in the presence of 10^{-5} M dexamethasone. c, chloramphenicol; ac, acetylated chloramphenicol.

Activating domain of NS-1. Defined regions of the NS-1 protein were fused with the DNA-binding domain of LexA to identify the functional domain(s) involved in transcription activation. As depicted in Fig. 1A, different amino- and carboxy-terminal fragments of NS-1 (from plasmids pSVLexNSN and pSVLexNSC, respectively) were more particularly tested, since previous results have shown both extremities of the protein to be involved in promoter inhibition and toxicity (39). A point mutation in the ATPase site of NS-1 (pSVLex3241), which abolishes its capacity for inducing promoter P38 (39), was also examined. In order to assess *trans*-regulating effects, each NS-1 mutant was compared with a construct that had a similar DNA sequence but failed to produce NS polypeptides. Plasmid pSVLexNSinv served as a negative control for pSVLex3241, while frameshifted derivatives of pSVLexNSN/C were obtained by filling in the unique *Mlu*I site of the LexA-coding sequence. Chloramphenicol acetyltransferase activities were measured after cell cotransfection with the pMMTV/4Lxo*cat* reporter and various quantities of either the NS-1 mutant or control plasmids.

The pSVLexNSC1 construct, which encodes a fusion protein comprising the 126 carboxy-terminal amino acids of NS-1, proved able to activate *cat* expression driven by the MMTV/4Lxo promoter (Fig. 4). This effect was dependent on the NS-1 fragment, since it was abolished by a frameshift mutation preventing this constituent from being synthesized (pSVLexNSC1fr). It therefore appears that the C-terminal sequence of NS-1 is sufficient to constitute a transcription-activating domain. As mentioned above for the whole NS-1 fusion protein (Fig. 3), dexamethasone (and hence active glucocorticoid receptors) was required to cooperate with LexNSC1 in the full induction of the modified MMTV promoter (data not shown). As illustrated in Fig. 4, similar levels of MMTV/4Lxo *trans* activation could be achieved by the NS-1 C-terminal fragment (pSVLexNSC1) and the complete protein (pSVLexNS), yet a higher effector plasmid concentration had to be used to optimize the effect of the truncated versus full NS-1 polypeptide. This is likely to be due, at least in part, to the reduced synthesis or stability of the former product, as demonstrated by Western blotting (data not shown). Moreover, the shift of the LexNSC1 versus LexNS-1 dose response may indicate that an additional region(s) besides C1 contributes to the overall *trans*-regulating capacity of the whole NS-1 protein. A possible candidate lies in the N-terminal portion of NS-1, which was found to reinforce the toxic activity of the carboxyl end (39), yet none of the N-terminal amino acid sequences

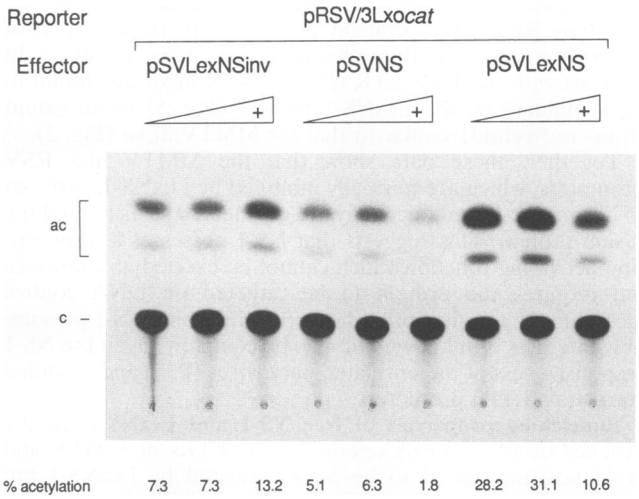


FIG. 5. NS-1 and LexNS-1 dose responses of a modified RSV promoter containing multiple LexA operators. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of the pRSV/3Lxo*cat* reporter plasmid and increasing quantities of the indicated effector plasmids (50, 500, and 5,000 ng of pSVLexNS or equimolar amounts of the other effector plasmids). c, chloramphenicol; ac, acetylated chloramphenicol.

tested (pSVLexNSN series of mutants) was able by itself to achieve a detectable *trans* activation in the LexA system (data not shown). Another region which appears to control the activating function of the whole NS-1 protein consists of the ATPase site, whose disruption (point mutation 3241) proved to abolish the inducing effects of NS-1 (39) and LexNS-1 (data not shown) on promoter P38 and MMTV/4Lxo, respectively. Since mutation 3241 apparently does not alter the stability of NS-1 (39), the region encompassing the ATPase site may control the activity of the C-terminal domain by affecting the overall structure of the protein. Alternatively, the acidic region may be unmasked in the LexNSC1 fusion protein but play a minor role in the genuine NS-1 product, whose activating function would involve, in particular, the ATPase domain. Although not ruled out by the present data, this possibility is undermined by the *trans*-activation deficiency of C-terminally deleted mutant NS-1 proteins (see above and reference 39) that keep the nucleoside triphosphate-binding region and the recently identified nuclear localization signals (48). The presence of a conventional activation domain in the full-size NS-1 product would also be consistent with the fact that *trans* activation depends on the association of this protein with target promoters (Fig. 4 and 5) and can be dissociated from its DNA helicase function (36).

The parvovirus MVM encodes two nonstructural proteins, NS-1 and NS-2, which share their 84 first amino acids but differ in their carboxyl end as a result of mRNA splicing (16). The NS component that is encoded by pSVLexNSC1 and is endowed with a *trans*-activating potential corresponds to the C-terminal sequence of NS-1. NS-2 appears to be inactive in this respect, as suggested by the above-mentioned inability of pSVLex3241 to *trans* activate the MMTV/4Lxo promoter. This clone contains an intact in-frame NS-2-coding sequence while being mutated in an NS-1-specific region that is spliced out of the NS-2 transcript (39). Together, these observations raise the question of which distinctive features provide the NS-1 carboxyl end with an activating capacity. It was previously noted that the C-terminal sequence of NS-1 possesses a net negative charge due to the clustering of 18 acidic amino acids, which led to speculation on the functional analogy of NS-1 with acidic transcriptional activators (39). In agreement with this possibility, a truncated NS-1 protein that lacks the 61 C-terminal amino acids, including 11 of 18 acidic residues, is impaired with regard to its ability to *trans* activate promoter P38 (39). In order to narrow down the C-terminal activating domain of NS-1, two subclones of pSVLexNSC1 were generated, pSVLexNSC2 and pSVLexNSC3, which contain the sequences coding for amino acids 592 to 672 and 547 to 589, respectively. These subclones proved unable to activate *cat* expression from plasmid pMMTV/4Lxo*cat* (data not shown), although the LexNSC2 fusion protein keeps 15 of the 18 acidic amino acids present in the LexNSC1 product. This result suggests that the integrity of the C1 domain of LexNSC1 is required for *trans* activation to occur. It is worth noting in this respect that 14 proline residues are found in the carboxy-terminal region of NS-1, which represents approximately 50% of the proline content of the protein (3). Moreover, 11 of these prolines are clustered in a 60-amino-acid sequence (residues 558 to 618) which is disrupted in the fragments encoded by pSVLexNSC2 and pSVLexNSC3. Given that proline-rich activation domains have been identified in some transcriptional factors (46), these residues may also contribute to the *trans*-regulating effect of the C-terminal domain of NS-1.

Targeting and *trans* activation of promoter P38 by NS-1. The P38 promoter of MVMp is a natural target of NS-1 which stimulates its activity and thereby positively controls the production of capsid protein (20, 51). NS-1 may associate with P38 either in a direct way or in the form of a complex with a cellular DNA-binding protein(s) serving as an adaptator(s) to target specific promoter motifs. Several upstream elements were found to be required in *cis* for the full induction of P38 by NS-1, in particular a tar region and a GC box (1, 30, 31, 52, 57). It is unclear whether these elements are involved in the direct or indirect binding of NS-1 and/or in the recognition of transcription factors that cooperate with NS-1 in the *trans* activation of the promoter. The LexA system allows one to distinguish between these possibilities. Indeed, a motif whose prime function is to directly or indirectly trap NS-1 should be replaceable by Lxo sequences, giving rise to a promoter that has a normal basal activity and is fully inducible by LexNS-1.

This hypothesis was tested by substituting four LexA operators for the tar element of P38 and measuring the activity of the modified P38/4Lxo promoter in the absence and presence of NS-1 or LexNS-1. As illustrated in Fig. 6, this substitution (i) had little influence on the basal P38 activity (lanes 1 and 3), (ii) decreased the sensitivity of the promoter to the *trans*-activating effect of the genuine NS-1 protein (lanes 6 and 8), and (iii) rendered P38 fully inducible by the LexNS-1 fusion polypeptide (lanes 11 and 13). The mere deletion of the tar element (P38/Δtar) conferred the first two properties but not the last property (Fig. 6, lanes 2, 7, and 12). Together, these features support the contention that tar is used for NS-1 targeting of promoter P38. Furthermore, a functional GC box was required to optimize the activity of unsubstituted P38 (Fig. 6, lanes 6, 9, 11, and 14) and p38/4Lxo (lanes 13 and 15) in the presence of (Lex) NS-1 and LexNS-1, respectively, as previously reported for the former promoter (30). LexNS-1 achieved a similar level of *trans* activation (about 100-fold) irrespective of whether the promoter contained an intact GC box (Fig. 6, lanes 1, 11, 3, and 13) or not (lanes 4, 14, 5, and 15). This suggests that NS-1 and GC box-binding factors may act independently to activate promoter P38.

It should also be stated that, as shown above for the MMTV promoter (Fig. 4), the carboxy-terminal C1 domain of NS-1 proved to be sufficient to activate promoter P38 under conditions of complex formation through the LexA system. Indeed, the pP38/4Lxo*cat* reporter was induced to a significant extent by cotransfected pSVLexNSC1, while this effect was abolished by a frameshift mutation (pSVLexNSC1fr) that prevented the NS fragment from being synthesized (Fig. 7). This observation therefore confirms that an activating domain is harbored by the C1 region of NS-1. As discussed in the previous section for the MMTV/4Lxo promoter, the P38/4Lxo-activating potency of LexNSC1 was lower than those of LexNS-1 and NS-1 at a given effector plasmid concentration, which may be attributed mainly to the lower level of accumulation of the truncated protein in transfected cells.

DISCUSSION

Activating function of NS-1. The results presented in this report show that promoters which are normally not stimulated by NS-1 can be *trans* activated by LexNS-1, provided that they are supplemented with LexA operators (Lxo). Together with the previous demonstration of the inducing effect of NS-1 at the RNA level (20, 38), these data indicate that the NS-1 protein can function as a transcriptional activator when bound to promoter sequences. An activation domain was found to be located in the 126 C-terminal amino acids of NS-1. When fused with the DNA-binding domain of LexA, this carboxyl end of NS-1 proved able to fully induce a Lxo-containing test promoter. It is noteworthy that the C-terminal region of NS-1 is

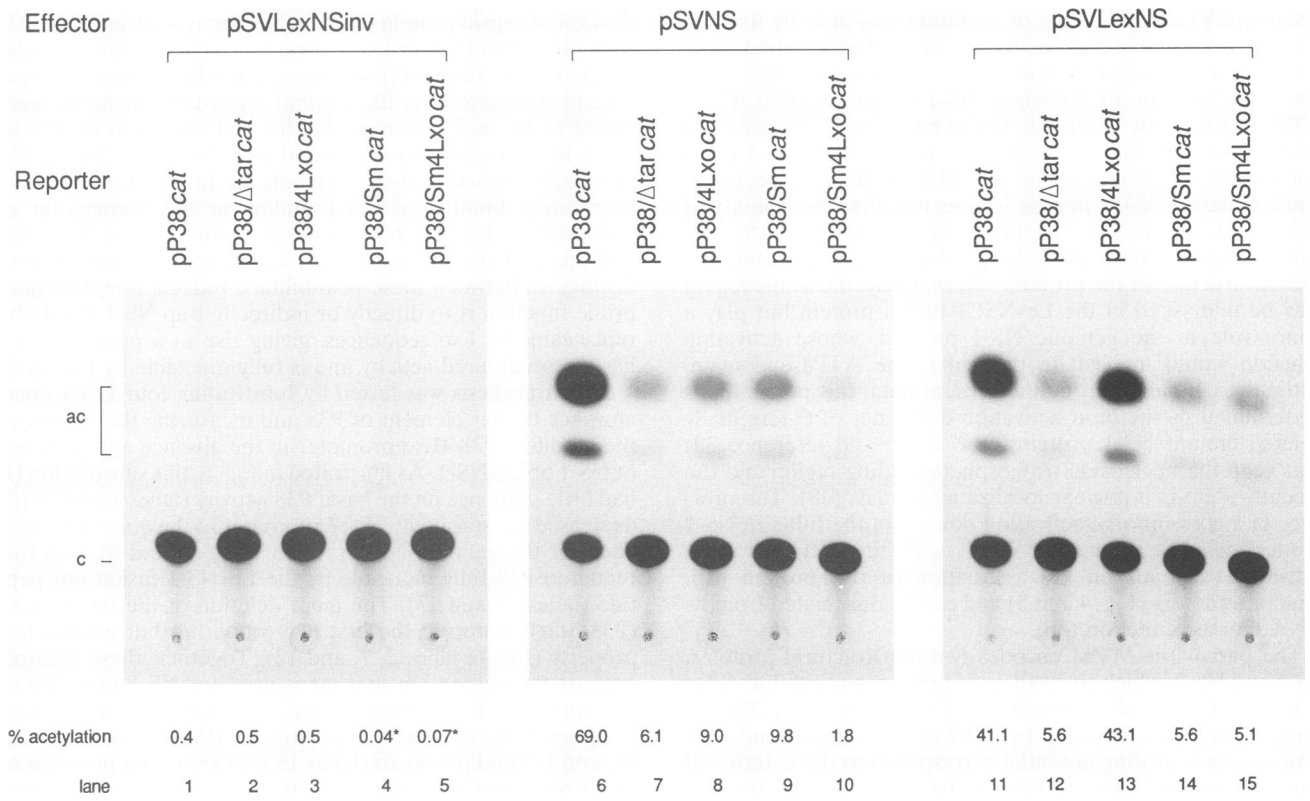


FIG. 6. Effects of tar replacement by LexA operators on the *trans*-activability of the P38 promoter by NS-1 and LexNS-1. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of reporter plasmid and 5 μg of pSVLexNS or an equimolar amount of the other effector plasmids. c, chloramphenicol; ac, acetylated chloramphenicol; ND, not detectable. *, determined from a 15-fold-larger amount of cell extract.

rich in proline and acidic residues, which are hallmarks of other transcriptional regulators (46, 50). Given the limited number of LexA–NS-1 fusions tested and the observed differences in the dose responses of the full and truncated proteins, the present results do not rule out that NS-1 sequences lying outside the carboxyl end may also play some role in activation. In agreement with this possibility, a truncated NS-1 protein lacking the 107 C-terminal amino acids was found to keep a residual capacity for P38 *trans* activation (39). It is noteworthy that the insertion of Lxo as a substitute for the tar element allowed the P38 promoter to be fully induced by the NS-1 activating region fused to the DNA recognition domain of LexA. This suggests that the binding of the LexA tag of LexNS-1 to Lxo DNA mimics a natural interaction leading to the association of NS-1 with tar, yet this association may not be sufficient for NS-1 to exert its activating function. Indeed, tar alone proved unable to confer NS-1 responsiveness on a heterologous promoter to which this element was transferred (52). This suggests that *trans* activation requires NS-1 to cooperate with cellular factors binding to other promoter sequences besides tar.

Promoter inhibition by NS-1. The MMTV and RSV promoters, which are activated by LexA-tagged NS-1 when targeted through LexA operators, are normally repressed by this protein in the absence of the LexA constituents. Whether NS-1 exerts a positive or a negative control on gene expression therefore appears to be governed by the ability of the protein to interact with the promoter sequences concerned. It should also be stated that the stimulation of responsive promoters by NS-1 or LexNS-1 decreases or even becomes inverted at high doses of transfected effector plasmid (reference 52 and this study). Together, these observations argue for imputing the inhibitory properties of NS-1 to unbound proteins that sequester a factor(s) essential for promoter activity (squelching phenomenon). A similar mechanism of promoter inhibition has been reported for other strong transcriptional activators, e.g., yeast GAL4, herpes simplex virus VP16, and steroid hormone receptors (25, 33, 45, 59).

Cytotoxicity of NS-1. It has been shown by means of wild-type and mutated clones of NS genes that NS-1 has a toxic function (6, 8, 39, 40) which cosegregates with the promoter-inhibiting activity of the protein (39). This observation raises the possibility that NS-1 may disturb host cells, at least in part, by disregulating the expression of essential cellular genes. In keeping with this hypothesis, some transcriptional activators jeopardize the survival of yeast and mammalian cells, which is imputed to the inhibition of endogenous gene expression by a squelching phenomenon (5, 24, 25, 28, 61, 63). The NS-1 sequences responsible for cytopathogenicity have been located in the polypeptide N- and C-terminal parts, which are both negatively charged and endowed with a toxic activity (39).

(i) The present demonstration of an activating domain in the carboxyl end of NS-1 is consistent with the interrelation of the regulatory and toxic functions of this protein. Similarly, some activation domains proved to strongly inhibit endogenous gene expression and to be cytotoxic in yeast cells, in the absence of other known functional regions (25, 63). These effects may be traced back to the trapping of transcription factors by free activators.

(ii) In contrast, the N-terminal part of NS-1, though cyto-

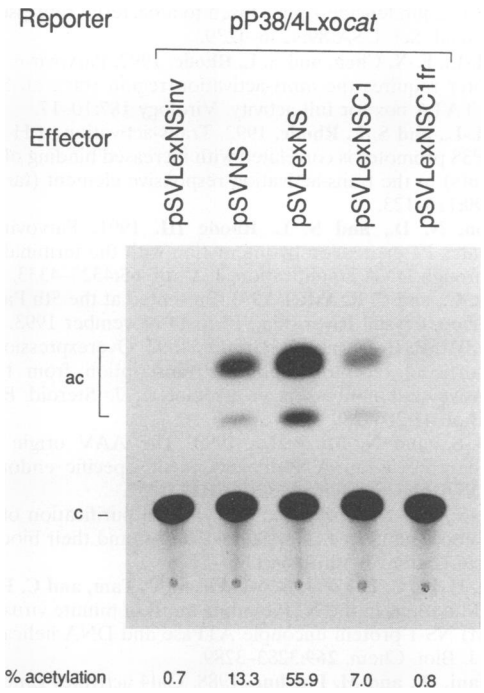


FIG. 7. *trans*-activating effects of wild-type and mutated LexNS-1 fusion proteins on a modified P38 promoter containing multiple LexA operators. Cultures (1.5×10^5 NBE cells) were cotransfected with 1 μg of the pP38/4Lxo*cat* reporter plasmid and 5 μg of pSVLexNS or an equimolar amount of the other effector plasmids. c, chloramphenicol; ac, acetylated chloramphenicol.

toxic on its own (39), failed to achieve a detectable *trans* activation in the LexA system. The mechanism by which the N fragment of NS-1 disturbs host cells is presently unclear. It has been reported that point mutations in this region impair the *trans*-activating capacity of NS-1 in a dominant fashion (54). This suggests that *trans* activation involves at least two NS-1 domains, one of which has an N-terminal location. Given that the activating function of NS-1 was mapped in the present work to the carboxyl end of the polypeptide, the N-terminal domain may be hypothesized to play a role in promoter recognition, either directly or through NS-1 interaction with a cellular adaptator protein(s). Should that be the case, the C- and N-terminal NS-1 domains could each contribute to cytotoxicity by sequestering essential cellular factors involved in transcription activation and DNA binding, respectively. The isolated N fragment might also form inactive complexes with target promoters. An action of that sort has been reported for VP16, a transcriptional regulator which is devoid of DNA-binding ability but can interact with some promoters through the octamer-binding protein Oct-1 (23, 56). Indeed, a truncated VP16 protein lacking the activation domain was found to dominantly inhibit responsive promoters (18, 59) and to exert a toxic activity in mammalian cells (18).

(iii) It should also be stated that the integrity of both the activation and promoter recognition regions is necessary for some transcriptional regulators to be fully inhibitory and cytopathic (5, 24, 49). This toxicity may be attributed to the trapping of transcription factors at many secondary genomic sites and/or to the inappropriate induction of target genes. Interestingly, the extremities of NS-1 also appear to act synergistically in the disturbance of host cells (39).

Conclusion. The data presented in this paper indicate that the NS-1 protein of MVMp activates gene expression driven by various promoters that either contain the NS-1-responsive tar region or are engineered so as to bind NS-1. The tar element is likely to act as a direct or indirect signal for the recognition of DNA by NS-1, since it can be replaced by LexA operators without impairing promoter induction by the LexNS-1 fusion protein. Promoters which are induced by NS-1 when recognized through the LexA system are inhibited by this protein in the absence of LexA-mediated targeting. These features argue for NS-1 being a transcriptional activator, given that proteins of this type can also exert an inhibitory action under conditions in which they fail to bind and/or activate the target promoter (squelching phenomenon). An activation domain rich in proline and acidic residues is located in the carboxyl end of NS-1. This domain appears to cooperate with the N-terminal fragment of the protein in the regulatory and cytotoxic functions of NS-1. The effects of NS-1 are probably mediated by interacting cellular proteins and are modulated as a result of cell transformation by oncogenes (47). This raises the intriguing possibility of using NS-1 as a probe to identify partner proteins that may constitute markers of neoplastic transformation.

ACKNOWLEDGMENTS

This work was supported by grants from the Commission of the European Communities, Caisse Générale d'Epargne et de Retraite, Services de la Programation de la Politique Scientifique, and Fonds de la Recherche Scientifique Médicale. D. Legendre was supported by the Televie action of the Fonds National de la Recherche Scientifique (Belgium).

ADDENDUM

The presence of a transcriptional activation domain within the C-terminal amino acids of NS-1 is further supported by two recent studies using NS-1 fused to the Gal4 DNA-binding region (32a, 37a).

REFERENCES

1. **Ahn, J. K., Z. W. Pitluk, and D. C. Ward.** 1992. 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. J. Virol. **66**:3776–3783.
2. **Astell, C. R., M. Thomson, M. B. Chow, and D. C. Ward.** 1983. Structure and replication of minute virus of mice DNA. Cold Spring Harbor Symp. Quant. Biol. **47**:751–762.
3. **Astell, C. R., M. Thomson, M. Merchlinsky, and D. C. Ward.** 1983. The complete DNA sequence of minute virus of mice, an autonomous parvovirus. Nucleic Acids Res. **11**:999–1018.
4. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61**:1199–1208.
5. **Berger, S. L., B. Pina, N. Silverman, G. A. Marcus, J. Agapite, J. L. Regier, S. J. Triezenberg, and L. Guarente.** 1992. Genetic isolation of ADA2: a potential transcriptional adaptator required for function of certain acidic activation domains. Cell **70**:251–265.
6. **Brandenburger, A., D. Legendre, B. Avalosse, and J. Rommelaere.** 1990. NS-1 and NS-2 proteins may act synergistically in the cytopathogenicity of parvovirus MVM(p). Virology **174**:576–584.
7. **Brent, R., and M. Ptashne.** 1984. A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene. Nature (London) **312**:612–615.
8. **Caillet-Fauquet, P., M. Perros, A. Brandenburger, P. Spegelaere, and J. Rommelaere.** 1990. Programmed killing of human cells by means of an inducible clone of parvoviral genes encoding nonstructural proteins. EMBO J. **9**:2989–2995.
9. **Cato, A. C. B., R. Miksicek, G. Schütz, J. Arnemann, and M. Beato.** 1986. The hormone regulatory element of mouse mammary

tumour virus mediates progesterone induction. EMBO J. **5**:2237–2240.

10. **Christensen, J., T. Storgaard, B. Viuff, B. Aasted, and S. Alexandersen.** 1993. Comparison of promoter activity in Aleutian mink disease parvovirus, minute virus of mice, and canine parvovirus: possible role of weak promoters in the pathogenesis of Aleutian mink disease parvovirus infection. J. Virol. **67**:1877–1886.
11. **Cotmore, S. F., J. P. F. Nüesch, and P. Tattersall.** 1992. In vitro excision and replication of 5′ telomeres of minute virus of mice DNA from cloned palindromic concatemer junctions. Virology **190**:365–377.
12. **Cotmore, S. F., J. P. F. Nüesch, and P. Tattersall.** 1993. Asymmetric resolution of a parvovirus palindrome in vitro. J. Virol. **67**:1579–1589.
13. **Cotmore, S. F., and P. Tattersall.** 1987. The autonomously replicating parvoviruses of vertebrates. Adv. Virus Res. **33**:91–173.
14. **Cotmore, S. F., and P. Tattersall.** 1988. The NS-1 polypeptide of minute virus of mice is covalently attached to the 5′ termini of duplex replicative-form DNA and progeny single strands. J. Virol. **62**:851–860.
15. **Cotmore, S. F., and P. Tattersall.** 1989. A genome-linked copy of the NS-1 polypeptide is located on the outside of infectious parvovirus particles. J. Virol. **63**:3902–3911.
16. **Cotmore, S. F., and P. Tattersall.** 1990. Alternate splicing in a parvoviral nonstructural gene links a common amino-terminal sequence to downstream domains which confer radically different localization and turnover characteristics. Virology **177**:477–487.
17. **Cotmore, S. F., and P. Tattersall.** 1992. In vivo resolution of circular plasmids containing concatemer junction fragments from minute virus of mice DNA and their subsequent replication as linear molecules. J. Virol. **66**:420–431.
18. **Dent, C. L., J. K. Estridge, L. M. Kemp, and D. S. Latchman.** 1990. Inhibition of histone H2B gene transcription and of cellular growth by a truncated viral *trans*-activator protein. Mol. Cell. Biol. **10**:3258–3261.
19. **Doerig, C., B. Hirt, J.-P. Antonietti, and P. Beard.** 1990. Nonstructural protein of parvovirus B19 and minute virus of mice controls transcription. J. Virol. **64**:387–396.
20. **Doerig, C., B. Hirt, P. Beard, and J.-P. Antonietti.** 1988. Minute virus of mice non-structural protein NS-1 is necessary and sufficient for *trans*-activation of the viral P39 promoter. J. Gen. Virol. **69**:2563–2573.
21. **Faisst, S. R., S. Faisst, C. Grangette, J. R. Schlehofer, and J. Rommelaere.** 1993. NFκB upstream regulatory sequences of the HIV-1 LTR are involved in the inhibition of HIV-1 promoter activity by the NS proteins of autonomous parvoviruses H-1 and MVMp. Virology **197**:770–773.
22. **Gavin, B. J., and D. C. Ward.** 1990. Positive and negative regulation of the minute virus of mice P38 promoter. J. Virol. **64**: 2057–2063.
23. **Gerster, T., and R. G. Roeder.** 1988. A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA **85**:6347–6351.
24. **Gilbert, D. M., D. M. Heery, R. Losson, P. Chambon, and Y. Lemoine.** 1993. Estradiol-inducible squelching and cell growth arrest by a chimeric VP16-estrogen receptor expressed in *Saccharomyces cerevisiae*: suppression by an allele of *PDR1*. Mol. Cell. Biol. **13**:462–472.
25. **Gill, G., and M. Ptashne.** 1988. Negative effect of the transcriptional activator Gal4. Nature (London) **334**:721–724.
26. **Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79**:6777–6781.
27. **Gorman, C. M., L. F. Moffatt, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
28. **Gossen, M., and H. Bujard.** 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc. Natl. Acad. Sci. USA **89**:5547–5551.
29. **Granger-Schnarr, M., E. Benusiglio, M. Schnarr, and P. Sassone-Corsi.** 1992. Transformation and transactivation suppressor activity of the c-jun leucine zipper fused to a bacterial repressor. Proc. Natl. Acad. Sci. USA **89**:4236–4239.
30. **Gu, M.-L., F.-X. Chen, and S. L. Rhode.** 1992. Parvovirus H-1 P38 promoter requires the *trans*-activation region (tar), an SP1 site, and a TATA box for full activity. Virology **187**:10–17.
31. **Gu, M.-L., and S. L. Rhode.** 1992. Trans-activation of H-1 parvovirus P38 promoter is correlated with increased binding of cellular protein(s) to the trans-activation responsive element (tar). Virology **190**:116–123.
32. **Hanson, N. D., and S. L. Rhode III.** 1991. Parvovirus NS1 stimulates P4 expression by interaction with the terminal repeats and through DNA amplification. J. Virol. **65**:4325–4333.

32a. **Harris, C., and C. R. Astell.** 1993. Presented at the 5th Parvovirus Workshop, Crystal River, Fla., 10 to 14 November 1993.

33. **Hoeck, W., P. Hofer, and B. Groner.** 1992. Overexpression of the glucocorticoid receptor represses transcription from hormone responsive and nonresponsive promoters. J. Steroid Biochem. Mol. Biol. **41**:283–289.
34. **Im, D.-S., and N. Muzyczka.** 1990. The AAV origin binding protein rep68 is an ATP-dependent site-specific endonuclease with DNA helicase activity. Cell **61**:447–457.
35. **Im, D.-S., and N. Muzyczka.** 1992. Partial purification of adeno-associated virus Rep78, Rep52, and Rep40 and their biochemical characterization. J. Virol. **66**:1119–1128.
36. **Jindal, H. K., C. B. Yong, G. M. Wilson, P. Tam, and C. R. Astell.** 1994. Mutations in the NTP-binding motif of minute virus of mice (MVM) NS-1 protein uncouple ATPase and DNA helicase functions. J. Biol. Chem. **269**:3283–3289.
37. **Kakidani, H., and M. Ptashne.** 1988. Gal4 activates gene expression in mammalian cells. Cell **52**:161–167.

37a. **Krady, J. K., and D. C. Ward.** Personal communication.

38. **Krauskopf, A., O. Resnekow, and Y. Aloni.** 1990. A *cis* downstream element participates in regulation of in vitro transcription initiation from the P38 promoter of minute virus of mice. J. Virol. **64**: 354–360.
39. **Legendre, D., and J. Rommelaere.** 1992. Terminal regions of the NS-1 protein of the parvovirus minute virus of mice are involved in cytotoxicity and promoter *trans* inhibition. J. Virol. **66**:5705–5713.
40. **Legrand, C., J. Rommelaere, and P. Caillet-Fauquet.** 1993. MVM(p) NS-2 protein expression is required with NS-1 for maximal cytotoxicity in human transformed cells. Virology **195**: 149–155.
41. **Little, J. W., and S. A. Hill.** 1985. Deletions within a hinge region of a specific DNA-binding protein. Proc. Natl. Acad. Sci. USA **82**: 2301–2305.
42. **Liu, Q., C. B. Yong, and C. R. Astell.** 1994. *In vitro* resolution of the dimer bridge of the minute virus of mice (MVM) genome supports the modified rolling hairpin model for MVM replication. Virology **201**:251–262.
43. **MacEwan, I. J., A. P. H. Wright, K. Dahlman-Wright, J. Carlstedt-Duke, and J.-A. Gustafsson.** 1993. Direct interaction of the τ_1 transactivation domain of the human glucocorticoid receptor with the basal transcriptional machinery. Mol. Cell. Biol. **13**:399–407.
44. **Merchlinsky, M. J., P. Tattersall, J. J. Leary, S. F. Cotmore, E. M. Gardiner, and D. C. Ward.** 1983. Construction of an infectious molecular clone of the autonomous parvovirus minute virus of mice. J. Virol. **47**:227–232.
45. **Meyer, M.-E., H. Gronemeyer, B. Turcotte, M.-T. Bocquel, D. Tasset, and P. Chambon.** 1989. Steroid hormone receptors compete for factors that mediate their enhancer function. Cell **57**:433–442.
46. **Mitchell, P. J., and R. Tjian.** 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science **245**:371–378.
47. **Mousset, S., Y. Ouadrhiri, P. Caillet-Fauquet, and J. Rommelaere.** 1994. The cytotoxicity of the autonomous parvovirus minute virus of mice nonstructural proteins in FR3T3 rat cells depends on oncogene expression. J. Virol. **68**:6446–6453.
48. **Nüesch, J. P., and P. Tattersall.** 1993. Nuclear targeting of the parvoviral replicator molecule NS-1: evidence for self-association prior to nuclear transport. Virology **196**:637–651.
49. **Prywes, R., and H. Zhu.** 1992. In vitro squelching of activated

transcription by serum response factor: evidence for a common coactivator used by multiple transcriptional activators. Nucleic Acids Res. **20:**513–520.

50. **Ptashne, M.** 1988. How eukaryotic transcriptional activators work. Nature (London) **335:**683–689.
51. **Rhode, S. L., III.** 1985. *trans*-activation of parvovirus P_{38} promoter by the 76K noncapsid protein. J. Virol. **55:**886–889.
52. **Rhode, S. L., III, and S. M. Richard.** 1987. Characterization of the *trans*-activation-responsive element of the parvovirus H-1 P38 promoter. J. Virol. **61:**2807–2815.
53. **Shein, H. M., and J. F. Enders.** 1962. Multiplication and cytopathogenicity of simian vacuolating virus 40 in cultures of human tissues. Proc. Soc. Exp. Biol. Med. **109:**495–500.
54. **Skiadopoulos, M. H., and E. A. Faust.** 1993. Mutational analysis of conserved tyrosines in the NS-1 protein of the parvovirus minute virus of mice. Virology **194:**509–517.
55. **Skiadopoulos, M. H., R. Salvino, W. L. Leong, and E. A. Faust.** 1992. Characterization of linker insertion and point mutations in the NS-1 gene of minute virus of mice: effects on DNA replication and transcriptional activation functions of NS-1. Virology **188:** 122–134.
56. **Stern, S., M. Tanaka, and W. Herr.** 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. Nature (London) **341:**624–630.
57. **Storgaard, T., J. Christensen, B. Aasted, and S. Alexandersen.** 1993. *cis*-acting sequences in the Aleutian mink disease parvovirus late promoter important for transcription: comparison with the canine parvovirus and minute virus of mice. J. Virol. **67:**1887–1895.
58. **Tenenbaum, L., F. Dupont, P. Spegelaere, L. Zentilin, P. Norio, M. Giacca, S. Riva, A. Falaschi, and J. Rommelaere.** 1993. Inhibition of heterologous DNA replication by the MVMp nonstructural NS-1 protein: identification of a target sequence. Virology **197:** 630–641.
59. **Triezenberg, S. J., K. L. LaMarco, and S. L. McKnight.** 1988. Evidence of DNA:protein interactions that mediate HSV-1 immediate early gene activation by VP16. Genes Dev. **2:**730–742.
60. **Vanacker, J.-M., V. Laudet, G. Adelmant, D. Stéhelin, and J. Rommelaere.** 1993. Interconnection between thyroid hormone signalling pathways and parvovirus cytotoxic functions. J. Virol. **67:**7668–7672.
61. **Werstuck, G., P. Bilan, and J. P. Capone.** 1990. Enhanced infectivity of herpes simplex virus type 1 viral DNA in a cell line expressing the *trans*-inducing factor VmW65. J. Virol. **64:**984–991.
62. **Wilson, G. M., K. J. Hitesh, D. E. Yeung, W. Chen, and C. R. Astell.** 1991. Expression of minute virus of mice non structural protein in insect cells: purification and identification of ATPase and helicase activities. Virology **185:**90–98.
63. **Wright, A. P. H., I. J. McEwan, K. Dahlman-Wright, and J.-A. Gustafsson.** 1991. High level expression of the major transactivation domain of the human glucocorticoid receptor in yeast cells inhibits endogenous gene expression and cell growth. Mol. Endocrinol. **5:**1366–1372.