Vaccinia virus early gene transcription termination requires the vaccinia termination factor (VTF), NPH I, a single stranded DNA-dependent ATPase, the virion form of RNA polymerase containing the Rap 94 subunit, and the signal UUUUUNU, which resides in the nascent mRNA, located 30 to 50 bases upstream from the poly(A) addition site. Evidence indicates that a required termination factor acts through binding to the UUUUUNU signal. To further investigate the function of UUUUUNU, the ability of UUUUUNU containing oligonucleotides to inhibit transcription termination was tested. A 22-mer RNA oligonucleotide containing a central U9 sequence exhibited sequence and concentration-dependent stimulation of premature transcription termination and transcript release, in trans. Activation of premature termination required VTF, NPH I, Rap 94, and ATP, demonstrating that the normal termination machinery was employed. Premature termination was not stimulated by RNA harboring a mutant UUUUUNU, demonstrating specificity. These data are consistent with a model in which a required termination factor is converted from an inactive to an active form by binding to a UUUUUNU containing oligonucleotide. The active termination factor then interacts with the ternary complex stimulating transcription termination through the normal mechanism, independent of the nascent mRNA sequence.

Poxviruses are double stranded DNA viruses that replicate in the cytoplasm of infected cells. To conduct this unusual life cycle, poxviruses encode the enzymes employed in viral gene transcription, mRNA processing, genome replication, and recombination (1). Vaccinia virus, a member of the poxvirus family, is the strain employed as smallpox vaccine. Poxvirus gene expression is divided into three temporal classes that differ in their promoter sequences and the protein factors employed in transcription initiation (2). Early genes are transcribed in the virion core by a virus-encoded multisubunit RNA polymerase (3) that contains the Rap 94 subunit (15–17), and VTF1 (18), the vaccinia termination factor composed of the 97-kDa D1R subunit (19) and the 33-kDa D12L subunit (20). VTF is also the virion mRNA capping enzyme employed in catalyzing the first three steps in cap formation (6, 21). In addition, ATPase activity catalyzed by nucleoside-triphosphate phosphohydrolase I (NPH I), the product of gene D11L, is essential for transcription termination and transcript release (22, 23). An interaction between the C-terminal end of NPH I and the N-terminal end of Rap 94 is required for termination (15–17, 24). Finally, termination also utilizes the sequence TTTTTNT present in the gene about 30 to 50 base pairs upstream from the map position of the early mRNA poly(A) addition site. The signal is recognized as UUUUUNU in the nascent mRNA (25). One appealing model proposes that the UUUUUNU signal is recognized by an undefined essential termination factor and upon binding, this factor initiates the termination/release sequence of events.

To further investigate the putative UUUUUNU recognition factor we employed RNA oligonucleotides containing UUUUUNU or a mutated sequence as potential inhibitors of transcription termination, in vitro. We hypothesized that binding of the UUUUUNU recognition factor to the UUUUUNU signal in the nascent mRNA would be inhibited in a sequence dependent fashion by the addition of a UUUUUNU containing oligonucleotide, resulting in an inhibition of termination. Analysis of RNA products synthesized in the presence of different concentrations of wild type and mutant UUUUUNU containing oligonucleotides showed that rather than observing an inhibition of termination, we found dramatically enhanced synthesis of truncated transcripts. In addition to the UUUUUNU containing oligonucleotide, the apparent premature termination required VTF, NPH I, Rap 94, and ATP or dATP, demonstrating that the normal viral termination machinery is employed. Oligonucleotide-dependent premature termination was independent of the TTTTTNT signal in the template and resulted in release of the termination products. Importantly, premature termination...
termienation required UUUUUNU in the activating oligonucleotide, thus retaining the essential signal requirement as found in vitro. This trans-activation of transcription termination demonstrates that the cis-acting signal present in nascent transcripts can be overridden by an exogenous oligonucleotide, in vitro. This observation impacts on the mechanism of early gene transcription termination and provides a novel site for the development of potential anti-poxvirus agents.

EXPERIMENTAL PROCEDURES

Cells and Viruses—Wild type (WT) vaccinia virus strain WR and the temperature-sensitive mutant virus, tsC50, were propagated in BSC40 African green monkey cells at 37 °C, or the permissive temperature for ts mutants, 31 °C, respectively, as described (26, 27). Virus titer was determined by plaque assay on BSC40 cells at the permissive temperature, 31 °C, and the nonpermissive temperature, 40 °C for tsC50 virus and at 37 °C for the WT virus.

Transcription Extracts—Transcription extracts were prepared from virus-infected cells by lysolecithin treatment, as described (25, 29). A549 cells were infected with either WT virus or tsC50 mutant virus at a multiplicity of infection of 15, at 37 or 31 °C, respectively. After 24 h, the medium was removed and replaced with 40 °C medium containing 100 μg/ml cycloheximide. After a further 24 h at 40 °C, cells were washed and treated with 250 μg/ml lysolecithin and extracts were prepared. In the case of the tsC50 virus-infected cells, this procedure permits the initial synthesis of active NPH I, which is required for intermediate and late gene expression. After switching to 40 °C, the endogenous NPH I is inactivated and cycloheximide prevents the synthesis of new protein.

Transcription Termination Assay—Construction of the G21(TER29)A78 and G21(TEK59)A78 templates was described (29), and the plasmids containing these sequences were generously provided by Dr. Stewart Shuman of the Memorial Sloan Kettering Cancer Center. The G21(TER29)A78 transcription unit contains a synthetic early promoter fused to a 20-nucleotide G-less cassette, which is followed by three G residues at positions +78 to +81. A termination signal, TTTTTTTTT, was placed downstream of the G-less cassette, spanning positions +29 to +37. Arrows represent the RNA products synthesized under various experimental reaction conditions. The lengths of the RNA products are noted on the right. B, biotin.

The bead-bound (B) template (typically, 100 fmol) was first incubated with 4 μl of C50 or WT virus-infected cell extracts, in the presence of 1 mM ATP, 10 μM of [α-32P]GTP (800 Ci/mmol), 0.1 mM UTP, and 0.625 mM 3′ O-Me-GTP to synthesize the G21 transcript. The ternary complex was isolated, washed twice with 0.5 ml of transcription salts, resuspended, and incubated in the presence or absence of VTF that was preincubated in the presence or absence of the oligo RNA for 10 min on ice, prior to incubation with the ternary complexes. Whenever mentioned, the ternary complexes were incubated in the presence or absence of IgG antibodies directed against different regions of the Rap 94 subunit of the virion RNA polymerase (the H4L protein), prior to their incubation with VTF and NPH I. Termination was then assessed after elongation of the ternary complex in the presence of 1 mM UTP, 1 mM GTP, 1 mM CTP, and 1 mM ATP. Elongation of the G21 RNA in the presence of all 4 NTPs to the end of the template, would yield a read-through transcript of about 177 bases in length. UUUUUNU-dependent termination would yield a termination product of about 70 bases in length. Greater than 90% of the isolated ternary complexes were routinely elongated in the second RNA synthesis reaction. RNA products were separated by gel electrophoresis, observed by autoradiography, and quantified by densitometry of the exposed film. Termination efficiency was calculated as the molar ratio of terminated RNA to the sum of read-through and terminated RNA.

Transcript Release from the G21 Ter 29 Complex—Bead-bound ternary complexes containing radiolabeled G21 RNA were constructed as described above using a tsC50 virus-infected cell extract. The ternary complex was isolated, washed, resuspended, and transcript release from the paused ternary complex was assessed in the presence or absence of VTF, NPH I, dATP, and oligonucleotides. After incubation for 10 min at 30 °C, the bound transcript was separated from the free RNA using a magnet, separated by gel electrophoresis, and analyzed as described above. To measure the time course of the G21 RNA release, G21 ternary complexes were prepared as described above using a tsC50 virus-infected cell extract. Reactions containing ternary complexes, NPH I, VTF, and dATP were set up and release was begun by the addition of the UUUUUNU oligonucleotide. At times after adding UUUUUNU, samples were taken and bound and free G21 RNA were separated using a magnet, and analyzed as described above.

RESULTS

Prior studies demonstrated that short single stranded oligonucleotides inhibit overall transcription in vitro, exhibiting a preferential inhibition of termination (31). The observed inhibition was seen with DNA or RNA and was independent of the UUUUUNU signal essential for early gene transcription termination. To evaluate the effect of UUUUUNU containing oligonucleotides on transcription termination separate from their effect on transcription initiation and elongation, bead-
bound templates were employed. Templates were described by the Shuman laboratory (29) (Fig. 1B), which permit initiation of transcription at a strong early promoter and elongation through a 20-nucleotide G-less cassette in the absence of GTP and the presence of 3′ O-MeGTP, yielding a 21-base RNA product referred to as G21 RNA. After isolation and washing the bead-bound ternary complex, elongation in the presence of all four nucleoside triphosphates and termination factors, NPH I (22, 23) and VTF (18), either full-length transcripts or termination products are formed, which can be separated by gel electrophoresis and observed by autoradiography. Two templates were prepared that differ in the location of the termination signal T9 (Fig. 1B). Ter 29 locates the T9 signal starting at position 29, and Ter 59 has the termination signal starting at position 59. The position of the termination signal determines the length of the termination product. Two RNA oligonucleotides were synthesized (Fig. 1A). The UUUUUUN oligonucleotide has a sequence identical to the nascent transcript synthesized from the Ter 29 template from base 21 to 42. In the mutant oligonucleotide, selected U residues were changed to A at positions 29, 30, 33, and 36 to yield an altered termination signal. The essential change is the A at position 33 that had been shown in the past to generate a sequence that was inactive in termination (32).

Transcription competent extracts were prepared from cells infected either with wild type virus, or with a mutant virus, tsC50, harboring a ts mutation in the D11L gene, under conditions that yield an extract with reduced NPH I activity. Both extracts are naturally deficient in VTF (23, 33) so maximum termination requires the addition of exogenous VTF, and in the case of C50 extract, both VTF and NPH I must be added. Transcription initiation and elongation to G21 permits the preparation of an isolated ternary complex and effectively separates transcription initiation from termination. The ternary complex can then be incubated with nucleoside triphosphates, oligonucleotides, and termination factors and the radiolabeled nascent RNA allowed either to elongate to the end of the template or to terminate in response to the UUUUUN signal.

The effect of RNA oligonucleotides containing a wild type or mutant termination signal on transcription termination was tested. Transcripts produced in the presence of increasing oligonucleotide concentrations were observed after separation by gel electrophoresis. In Fig. 2A, lane 2, the major transcript produced in the absence of added VTF or oligonucleotide is the full-length read-through transcript (RT). Minor shorter products (P), including a collection of pause products at the T9 sequence (30), and the un-elongated G21 RNA (lanes 1 and 10) can be identified. Minimal termination (Term) can be seen because of the low level of endogenous VTF. When VTF is added, lane 3, there is a decrease in RT and an increase in Term, as expected when transcription is conducted with a wild type virus-infected cell extract (23, 33). Upon the addition of
Oligonucleotide Stimulation of Transcription Termination

VTF plus increasing concentrations of a 22-base RNA containing the U9 termination signal (lanes 4–9) one observes a decrease in Term reflecting an inhibition of transcription termination at the normal site. Rather than finding a corresponding increase in RT, however, there is a dramatic increase in the level of short transcripts (PT). The concentration of UUUUUUN oligonucleotide required for half-maximal PT production is about 2 nM. Titration of a 22-base RNA possessing a mutant termination signal exhibits a minor reduction in Term with a minor increase in shorter RNA products (Fig. 2B). Control studies showed that oligonucleotide addition to the virus-infected cell extracts did not stimulate nuclease activity (data not shown). These results demonstrate that addition of an oligonucleotide containing the U9 sequence directs the signal-dependent stimulation of premature transcription termination, in trans.

To determine whether this phenomenon was template-dependent, the effect of oligonucleotide addition on transcription termination was evaluated using both the Ter 29 and Ter 59 templates. In Fig. 3, A and B, transcription elongation of G21 RNA was carried out in the absence or presence of VTF, and the oligonucleotides. In the absence of added factors, there is minimal termination (lane 2). Upon the addition of VTF (lane 3), there is an increase in termination, and as expected, the termination product produced from the Ter 59 template is longer than that synthesized from Ter 29. Addition of the U5NU oligonucleotide but not the mutant oligonucleotide results in a decrease in the normal termination product and an increase in the synthesis of the prematurely terminated RNA from both templates (lanes 4 and 5). Each template yielded similarly sized premature termination products demonstrating that the ability of this oligonucleotide to direct the synthesis of short RNA is not template dependent.

To assess whether VTF was required for oligonucleotide-directed stimulation of premature termination, the following study was conducted. RNA synthesis was carried out in the absence or presence of VTF and the U5NU oligonucleotide. In Fig. 4A, lane 1, one observes primarily the synthesis of readthrough RNA in the absence of added factors. When VTF is added (lane 3), the Term RNA accumulates. If the U5NU oligonucleotide is added in the absence of VTF, there is no change (lane 2). However, if the U5NU RNA is added in the presence of VTF (lane 4) the accumulation of the premature termination...
products is observed. When the VTF concentration is increased in the presence of the USNU RNA (Fig. 4B) there is a decrease in the synthesis of the read-through RNA and a marked increase in the synthesis of premature termination products. At high levels of VTF there is a reappearance of a low level of the normal termination product (Term), whereas the level of prematurely terminated RNA varies little. The normal termination products are formed from ternary complexes that escape prematurely terminated RNA varies little. The normal termination product, Term (lane 2), is added in the presence of added factors, the read-through RNA and pause products were observed (lane 2). Addition of VTF alone had little effect (lane 3) because the extract has low NPH I activity. Addition of VTF and NPH I resulted in the appearance of the normal termination product (lane 4). As in the case of VTF, addition of increasing levels of NPH I results in enhanced premature termination, Fig. 5B.

Early gene transcription termination was shown to require the N-terminal region of the Rap 94 subunit, the product of gene HAL (15, 16, 24). Antibodies that bind to the N-terminal end of Rap 94 prevent termination in vitro. A requirement for Rap 94 for premature termination was tested (Fig. 6). In this case, a tsC50-infected cell extract was employed that was deficient in both VTF and NPH I activity. In the absence of added factors, the read-through RNA and pause products were observed (lane 2). Addition of VTF alone had little effect (lane 3) because the extract has low NPH I activity. Addition of VTF and NPH I resulted in the appearance of the normal termination product, Term (lane 4). Addition of UUUUUNU along with VTF and NPH I (lane 5) caused the accumulation of the PT products. As shown previously (16), preincubation of the isolated ternary complexes with antibodies raised against the N-terminal but not the C-terminal region of Rap 94 prevented UUUUUNU oligonucleotide stimulation of premature termination (compare lanes 6 and 7).

Early gene transcription termination requires energy generated by NPH I-catalyzed ATP hydrolysis (22, 23). To evaluate
the energy requirement for U5NU-stimulated premature termination the standard transcription protocol was modified. Radiolabeled G21 RNA containing ternary complexes were prepared. Elongation through the A-less cassette to A78 was carried out in the absence of added ATP, and the presence or absence of dATP or cordycepin triphosphate. In Fig. 7A, lanes 1 and 2, display RNA was made in the presence of VTF, and the presence or absence of U5NU RNA. One observes the read-through RNA because of low levels of contaminating ATP. A prominent RNA product labeled A78 is formed because of pausing near A78 in the absence of added ATP. A78 migrates near the normal termination product, Term, which would be minimal in this assay because of the low level of ATP in these reactions (30). When cordycepin triphosphate is added along with VTF (lanes 3, 4, 7, and 8), one observes a failure to synthesize the read-through RNA because of the incorporation of the chain terminating base at A78. Addition of U5NU (lanes 4 and 8) stimulates premature termination indicating that cordycepin triphosphate can serve as an energy source. If dATP is added in the absence of cordycepin triphosphate (lane 5) one observes a failure to synthesize the read-through product. When U5NU is added (lane 6), premature termination products are observed demonstrating that dATP can serve as the energy source required for premature transcription termination. Addition of both cordycepin triphosphate and dATP has no additional effect (lanes 7 and 8).

To determine whether the newly synthesized RNA products are released from the ternary complexes, after elongation, bound and free RNA were separated and analyzed by gel electrophoresis. In Fig. 7B one observes that in the absence of added ATP, the RNA remains bound in the ternary complex whether U5NU is added or not (lanes 1–4). If an energy source is provided in the form of cordycepin triphosphate or dATP, the majority of the RNA products are released, A78, Term, and the premature termination products (lanes 5–16). Release occurs in the absence of added UUUUUNU oligonucleotide because of the fact that the Ter 29 template was employed that directs the synthesis of nascent RNA with an available UUUUUNU signal. Addition of the UUUUUNU oligonucleotide stimulates release in each case.

To ensure that the endogenous U5NU does not contribute to the oligonucleotide-dependent stimulation of transcript release and to determine whether an extended RNA 5′ tail is needed, the ternary complex stalled at position G21 was employed in the transcript release assay because this complex was certain to possess a short nascent transcript that lacks U5NU. Ternary complexes were constructed using a tsC50 mutant virus-infected cell extract. Incubations of the isolated, washed ternary complexes were conducted in the absence or presence of NPH I, VTF, and dATP, in the absence or presence of oligonucleotides that contained either a wild type or a mutated UUUUUNU termination signal (Fig. 8A). When dATP is omitted (lanes 1–4) there is little release of G21 RNA because of the lack of an energy source. If VTF is omitted (lanes 5–8) there is a modest stimulation of release (16%) when UUUUUNU RNA is added because of the low level of endogenous VTF in this assay. The
mutant oligonucleotide has a much lower effect on release (lanes 7 and 8). If NPH I is omitted (lanes 11–14) a low level of transcript release is observed that is higher (22%) when the UUUUUNU oligonucleotide is added rather than the mutant oligonucleotide (10%). If oligonucleotide is not added (lanes 9 and 10) only 20% release is observed. However, in the presence of VTF, NPH I, and dATP a high level (57%) of G21 RNA release can be observed in the presence of 2′-dATP, VTF, NPH I, or mutant UUUUUNU-containing oligonucleotides. B, the ternary complexes were incubated in the presence of 2′-dATP, VTF, NPH I, and UUUUUNU oligonucleotide. The bead-bound G21 transcripts (B, bound) were separated from the released G21 transcripts (F, free) by magnetic separation and analyzed by electrophoresis through a 12% polyacrylamide gel containing 8 M urea. The labeled G21 transcripts were visualized by autoradiography. The percent of RNA released, indicated below the autoradiograph, was quantified by scanning the autoradiogram with a densitometer.

DISCUSSION

A current model for termination of vaccinia virus early gene transcription describes the interplay of the nascent mRNA, the virion RNA polymerase, VTF, NPH I, and the template DNA (34). NPH I is a single stranded DNA-dependent ATPase (35, 36) that provides energy needed for transcript release (22, 23). The requirement for single stranded DNA for adoption of the active conformation indicates that NPH I is able to bind to DNA in an accessible region in the transcription bubble. Furthermore, studies demonstrated that the C-terminal end of NPH I must bind to the N-terminal domain of the RNA polymerase Rap 94 subunit (15, 16, 24), the product of gene H4L. Loss of NPH I binding or antibody binding to Rap 94 prevents termination, 
in vitro. A role for VTF, the first termination factor to be identified (18), has not been defined. However, VTF is clearly required for transcript release, 
in vitro (29).

The sequence UUUUUNU located upstream from the poly(A) addition site is essential for termination 
in vitro (32). Alteration of any U residue results in a reduction in termination efficiency. The UUUUUNU sequence resides 30 to 50 bases upstream from the 3′ end (37). This location places the UUUUUNU signal outside of the RNA polymerase in a region of the nascent mRNA that is not protected by the RNA polymerase from nuclease digestion (29). These two facts support a model in which the UUUUUNU sequence binds to an essential termination factor as a required step in transcription termination. Although VTF is a likely candidate, to date, there is no evidence to support this proposition.

These studies were undertaken to test the hypothesis that a UUUUUNU recognition factor binds to UUUUUNU and participates in early gene transcription termination. Addition of exogenously added RNA containing a U9 sequence was expected to inhibit normal transcription termination by binding to the termination factor and preventing its association with the UUUUUNU sequence in the nascent transcript. A sequence-dependent reduction in the normal termination product was seen, as anticipated. Unexpectedly, the accumulation of short transcripts was also observed. Premature termination was not determined by the template because it was found when either the Ter 29 or Ter 59 templates were used. In addition, premature termination required not only the sequence UUUUUNU in theactivating oligonucleotide but also VTF, NPH I, the N-terminal domain of Rap 94 and dATP, demonstrating that the normal transcription termination machinery was employed. Fi-
nally, UUUUUNU RNA was shown to stimulate the release of the G21 transcript from the isolated ternary complex. Because it was shown that RNA polymerase protects 18 bases of the nascent transcript from nuclease digestion, and the G21 RNA is not available for cap formation, in vitro (29, 38), UUUUUNU oligonucleotide-dependent transcript release must not require an extensive interaction with the 5' tail of the target RNA.

The premature termination products vary somewhat in length from experiment to experiment. This is because of the variable efficiency of release factor stimulation. Although release of G21 RNA in the absence of elongation is efficient (Fig. 8), in practice, there is a competition between elongation and release, in vitro.

Hybrid formation between the U9 oligonucleotide and either the nascent transcript or the template is not likely to contribute to premature termination. The nascent transcript lacks an oligo A sequence that would permit hybrid formation. The template strand has an A9 sequence, which could be a potential target for hybrid formation. However, G21 RNA is efficiently released from a ternary complex that has not reached the A9 sequence in the Ter 29 template. In addition, efficient premature termination is also observed with the Ter 59 template where the A9 sequence lies in a different sequence context. Finally, a DNA oligonucleotide of the same sequence, where T is substituted for U, which would be fully capable of hybrid formation, has no effect on premature termination, in vitro.

The most parsimonious model proposes that a UUUUUNU containing RNA oligonucleotide can bind to an inactive transcription termination factor and convert it to the active form. When activated, the termination factor initiates a sequence of events that results in release of the nascent RNA from the ternary complex. Normally, the activating UUUUUNU is present in the nascent RNA. This demonstrates that the newly formed transcript must be scanned by the recognition factor as transcription elongation proceeds. Because the termination occurs within 30 bases of the UUUUUNU sequence, scanning must be quite efficient. When the UUUUUNU signal is added in trans, however, the nascent transcript is disregarded and efficient transcript release is obtained without concomitant transcription elongation. Titration of the UUUUUNU containing RNA oligonucleotide exhibits a half-activation concentration of about 2 nM demonstrating a remarkably strong binding. RNA oligonucleotide-dependent transcript release does not require an RNA template-dependent transcript release, and the G21 RNA is not released, even when it is replaced simply by further addition of excess VTF or NPH I. UUUUUNU directly stimulates RNA release from about 80% of the G21RNA ternary complexes (Fig. 8B). Moreover, addition of UUUUUNU oligonucleotide to an ongoing transcription reaction results in near complete release of the nascent transcripts (Fig. 7B). These observations indicate that the ternary complexes are heterogeneous in regard to their ability to respond to normal and UUUUUNU-directed transcription termination. An understanding of the differences among the ternary complexes would provide information important to our understanding of the mechanism of early gene transcription termination.

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**REFERENCES**

1. Moss, B. (2001) in *Poxviridae: The Viruses and Their Replication*; Virology (Knipe, D. M., Howley, P. M., Griffin, D. E., Martin, M. A., Lamb, R. A., Roizman, B., and Strauss, S. E., eds) Vol. 2, pp. 2849–2883, Lippincott-Raven, Philadelphia.

2. Moss, B., Ahn, Y. B., Amegadzie, B., Gershun, P. D., and Keck, J. G. (1991) J. Biol. Chem. 266, 1355–1358.

3. Ensinger, M. J., Martin, S. A., Paoletti, E., and Moss, B. (1980) J. Biol. Chem. 255, 4372–4380.

4. Ahn, Y. B., and Moss, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3536–3540.

5. Kane, E., and Shuman, S. (1992) J. Virol. 66, 5752–5762.

6. Wei, C. M., and Moss, B. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3014–3018.

7. Kates, J. R., and Reeson, J. (1970) J. Mol. Biol. 50, 19–33.

8. Vos, J. C, Sasaki, M., and Stunnenberg, H. G. (1991) EMBO J. 10, 2553–2558.

9. Rosales, R., Harris, N., Ahn, Y. B., and Moss, B. (1994) J. Biol. Chem. 269, 14260–14267.

10. Keck, J. G., Baldick, C. J., Jr., and Moss, B. (1990) Cell 61, 811–809.

11. Gunasinghe, S. K., Hubbs, A. E., and Wright, C. F. (1998) J. Biol. Chem. 273, 27524–27530.

12. Rosales, R., Sutter, G., and Moss, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3794–3798.

13. Wright, C., Oswald, B., and Dellsis, S. (2003) J. Biol. Chem. 278, 46060–46068.

14. Böhrman, G., Yuen, L., and Moss, B. (1986) Cell 46, 1029–1035.

15. Mohamed, M. R., and Moss, B. (1990) J. Virol. 25798–25804.

16. Mohamed, M. R., and Niles, E. G. (2001) J. Biol. Chem. 276, 20758–20765.

17. Christen, L., Sanders, M., and Niles, E. G. (2002) Virology 309, 142–153.

18. Shuman, S., Broyles, S. S., and Moss, B. (1987) J. Biol. Chem. 262, 12372–12380.

19. Morgan, J. R., Cohen, L. K., and Roberts, B. E. (1984) J. Virol. 52, 283–297.

20. Niles, E. G., Chen, G. J., Shuman, S. M., and Broyles, S. S. (1989) Virology 172, 513–522.

21. Ensinger, M. J., Martin, S. A., Paolotti, E., and Moss, B. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2525–2529.

22. Deng, L., and Shuman, S. (1998) Genes Dev. 12, 538–546.

23. Christen, L. M., Sanders, M., Wiler, C., and Niles, E. G. (1998) Virology 245, 360–371.

24. Placente, S. C., Christen, L. M., Mohamed, M. R., and Niles, E. G. (2002) Virology, in press.

25. Shuman, S., and Moss, B. (1989) J. Biol. Chem. 264, 21536–21539.

26. Condit, R. C., and Motyczka, A. (1981) Virology 113, 224–241.

27. Condit, R. C., Motyczka, A., and Spiziz, G. (1983) Virology 172, 429–443.

28. Condit, R. C., Lewis, J. I., Quinn, M., Christen, L. M., and Niles, E. G. (1996) Virology 218, 169–180.

29. Deng, L., Hagler, J., and Shuman, S. (1996) J. Biol. Chem. 271, 19556–19562.

30. Hagler, J., Loo, Y., and Shuman, S. (1994) J. Biol. Chem. 269, 10050–10060.

31. Christen, L., Sanders, M., and Niles, E. G. (1999) Biochemistry 38, 8073–8089.

32. Yuen, L., and Moss, B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6417–6421.

33. Condit, R. C., Xiang, Y., and Lewis, J. I. (1996) Virology 230, 10–19.

34. Condit, R. C., and Niles, E. G. (2002) Biochem. Biophys. Acta 1577, 325–336.

35. Paolotti, E., and Moss, B. (1974) J. Biol. Chem. 249, 3281–3286.

36. Paolotti, E., Rosemond-Horneah, H., and Moss, B. (1974) J. Biol. Chem. 249, 273–280.

37. Yuen, L., and Moss, B. (1986) J. Virol. 60, 320–323.

38. Hagler, J., and Shuman, S. (1992) Science 255, 893–896.

39. Higman, M. A., Christen, L. A., and Niles, E. G. (1994) J. Biol. Chem. 269, 14974–14981.

40. Myette, J. R., and Niles, E. G. (1996) J. Biol. Chem. 271, 11936–11944.

41. Luo, Y., and Shuman, S. (1995) J. Biol. Chem. 268, 21253–21262.