Minute Virus of Mice Infection Modifies Cellular Transcription Elongation

ANAT KRAUSKOPF,[†] EDNA BEN-ASHER,^{*} AND YOSEF ALONI[‡]

Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot, Israel 76100

Received 31 August 1993/Accepted 23 December 1993

Our previous observations indicated that upon infection with minute virus of mice (MVM), Ehrlich ascites cells lose a transcription elongation activity which is essential for the readthrough of the MVM attenuator. This was monitored by the ability of extracts from uninfected but not from infected cells to support readthrough of the P4 attenuator when added to partially purified transcription elongation complexes. We have investigated the nature of this change in transcription elongation following MVM infection. In this communication, we show that infection of Ehrlich ascites cells with MVM leads to a general shift in the length of nascent mRNA synthesized in isolated nuclei and separated by sucrose gradients. Furthermore, infection leads to attenuation of transcription of the cellular gene *c-fos* but not *c-myc*. We show biochemical evidence to support a model by which, following MVM infection, there is a functional reduction in the activity of a TFIIS-like general transcriptional elongation activity.

Transcription mediated by eukaryotic RNA polymerase II can pause or terminate both *in vivo* and *in vitro* within viral (1, 2, 15, 16, 18, 25, 30–32, 35) and cellular (4, 6, 7, 13, 17) genes, thereby modulating downstream gene expression. This mechanism resembles attenuation in prokaryotes (37). To date, however, little is known about *cis*- and *trans*-acting elements involved in the attenuation mechanism in eukaryotes.

One of the model systems in our attenuation studies has been the parvovirus minute virus of mice (MVM). MVM has a linear single-stranded DNA genome of 5,149 nucleotides (nt). It is an autonomous virus but is dependent for replication upon cellular functions that are expressed during S phase (8, 11, 12, 22, 34). Transcription initiation on the double-stranded replicative form catalyzed by the host RNA polymerase II maps to two MVM promoters: P4, located at nt 201 ± 5, and P38, located at nt 2005 ± 4 (2, 10, 20, 28). Our earlier work demonstrated that *in vivo* and *in vitro*, RNA polymerase II attenuates transcription at a specific location 142 to 147 nt downstream from the P4 promoter late in MVM infection (2, 30). Additionally, salt-soluble factors were implicated in the process of transcription attenuation (5, 19, 30).

An essential step toward understanding the factors regulating the elongation block at the molecular level is the development of an *in vitro* system that mimics the *in vivo* situation. Toward this aim, we have developed a promoter-dependent assay during which brief initiation of transcription takes place in crude cellular extracts. Elongation complexes are then partially purified by gel filtration chromatography at high KCl concentrations. The column-purified transcription complexes, which are depleted of elongation factors, serve as a model system for determining the activities of factors acting during elongation (5). When this procedure was applied to the study of factors which modulate attenuation at the MVM attenuator, the results indicated that upon infection with MVM, Ehrlich ascites (EA) cells lose an elongation activity which is essential for the readthrough of the MVM attenuator (19). The resulting elongation block was relieved by addition of the known

general elongation factors or by extracts from uninfected but not from MVM-infected EA cells.

In the present study, we initially asked whether infection of EA cells by MVM leads to an overall shortening of the RNA synthesized. The rationale of this assumption was that perhaps the activity modified in infection is a general one, namely, one which affects the recognition of different pause or attenuation signals in addition to that of the MVM attenuator. In this case, the activity's decrease might affect cellular transcription, leading to an overall shift in the size distribution of newly synthesized mRNA. To this end, we performed a run-on experiment (as described by Greenberg and Ziff [14]) in which nuclei were isolated from EA cells 24 h after MVM infection or from uninfected EA cells with Nonidet P-40 lysis buffer. Nascent transcripts were labeled for 7 min at 29°C, after which reactions were stopped and RNA was extracted and precipitated as described previously (14), with the exception that no NaOH treatment was performed. RNA was warmed for 10 min at 37°C in 90% formamide and diluted in sodium dodecyl sulfate (SDS) buffer, as described by Laub et al. (21). RNA was separated on linear 15 to 30% sucrose gradients. Fractions were collected from the bottom of the gradient, and their radioactivity was counted. The amount of labeling in each area of the gradient reflects the abundance of RNA molecules of a certain size that were in the process of synthesis at the time of isolation of the nuclei. Transcription reactions were performed in the absence or presence of 2 µg of α -amanitin per ml. Results in Fig. 1A are represented as the amount of α -amanitin-sensitive transcription (reflecting RNA polymerase II-transcribed RNA), calculated by subtracting the α -amanitin-insensitive portion (panel B) from the total amount incorporated in each fraction. As can be seen in Fig. 1A, following viral infection, there was an overall decrease in the length of the newly transcribed RNA, reflected by the shift in RNA distribution toward the top of the gradient (fractions 40 to 80) and a corresponding decrease in the amount of RNA at the bottom of the gradient (fractions 1 to 40). This implies that there is a higher density of RNA polymerase II molecules engaged in transcription at the regions which are closer to the transcription initiation sites in infected cells. It is important to mention that viral RNA accounts for about 0.15% of the total RNA synthesized in the cells at this stage of infection (9);

^{*} Corresponding author. Phone: 972-8-342208. Fax: 972-8-344108.

[†] Present address: Department of Microbiology and Immunology, University of California, San Francisco, Calif.

[‡] Deceased.

FIG. 1. Overall size decrease of newly transcribed RNA produced by polymerase II following MVM infection. (A) Polymerase II RNA (as calculated by subtracting α -amanitin-insensitive counts [panel B] from the total counts along the gradient for each treatment) newly transcribed from uninfected nuclei and from infected nuclei 24 h postinfection was separated on 15 to 30% sucrose gradients in SDS buffer by centrifugation at 25,000 rpm for 16 h at 20°C in a Beckman SW-27.1 rotor. The top of the gradient is indicated. (B) α -Amanitin-insensitive newly transcribed RNA (10^6 cpm) from uninfected nuclei and infected nuclei 24 h postinfection was separated on linear 15 to 30% sucrose gradients as described in the legend to panel A.

therefore, its contribution to the transcription pattern of infected cells is negligible. In light of the generality of the effect of MVM infection on transcription of both the cellular precursor RNAs (this paper) and the viral RNA (19, 30), we hypothesized that this block was due to a modification of a general transcription elongation activity induced by the viral infection. This modification resulted in the accumulation of shorter nascent mRNA transcripts. The general transcriptional activity of infected cells was as high as that of uninfected cells, as evidenced by the fact that the total amounts of labeled RNA were similar in infected and noninfected nuclei, and we wanted to verify whether the accumulation of shorter mRNAs was due to specific blocks to transcription elongation at discrete sites within some cellular genes.

We chose to analyze the cellular genes *c-fos* and *c-myc*, since they have been reported to be regulated at the level of

FIG. 2. Transcription elongation of *c-fos* but not of *c-myc* is attenuated following MVM infection as monitored by run-on analysis. (A) Nascent RNA from infected (I) and uninfected (U) nuclei was hybridized to 5' (–459 to +540 relative to the promoter; *SacI*-*XhoI* fragment) and 3' (+540 to +2548; *XhoI*-*SacI* fragment) regions of the *c-fos* gene (lanes 1 and 2) and to 5' (–1950 to +914 relative to the P1 promoter; *HindIII*-*SacI* fragment) and 3' (+1520 to +3065; *SacI*-*SacI* fragment) regions of the *c-myc* gene (lanes 3 and 4) and to a control gene, GAPDH (lanes 5 and 6). p BSC is the plasmid DNA (pBlue-script; Stratagene) into which each of the other probes is inserted; thus it is a control for background hybridization (lanes 7 and 8). (B) Unlabeled RNA from infected (I) and uninfected (U) isolated nuclei was Northern blotted and hybridized to a GAPDH DNA probe.

transcriptional elongation (24, 26). A block to transcription elongation at the *c-myc* attenuator is induced when HL-60 cells were driven to differentiate (6). In the case of the *c-fos* gene, attenuation was observed in hamster fibroblasts when driven to differentiate (13). In both cases, the block to transcription elongation occurs at specific sites that were identified both in vivo and in vitro. We therefore performed transcription in nuclei isolated from infected and uninfected EA cells. RNA was labeled for 5 min, purified as described by Greenberg and Ziff (14) except for an additional NaOH treatment followed by neutralization with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) before the second ethanol precipitation, and hybridized to filters (14) carrying 5' and 3' DNA portions of the cellular genes *c-fos*, *c-myc*, and, as a control, GAPDH (glyceraldehyde-3-phosphate-dehydrogenase). The GAPDH gene is expressed at similar rates in different tissues (27) and therefore is used as a control for total transcription activity and RNA recovery (25). The results shown in Fig. 2A indicate that the amount of labeled RNA which hybridized to the 5' portion of the mouse *c-fos* gene relative to that which hybridizes to the 3' portion is increased in the infected nuclei (lane 2) compared with the uninfected nuclei (lane 1). However, *c-myc* transcription elongation, as seen in this experiment, was not altered and the 5'/3' RNA hybridization ratio remained constant (lanes 3 and 4). The intensity of GAPDH transcription, which is not regulated at the elongation level, remained unchanged in infected cells (Fig. 2A, lane 6) compared with that of uninfected cells (lane 5), indicating that the general efficiency of transcription was unaffected by MVM infection. The possibility that the differences between infected and noninfected nuclei result from some specific degradation process in infected cells was checked by performing unlabeled transcription in isolated nuclei and running the RNA through a formaldehyde-agarose gel (14). The gel was Northern (RNA) blotted, as described previously (14), and hybridized to a GAPDH probe. As can be seen in Fig. 2B, the sizes of the GAPDH transcripts were

similar in infected and uninfected nuclei, and no major degradation was observed. It should be noted that *c-fos* was attenuated in these cells even prior to the viral infection, as can be seen by the calculated ratio (normalized to the number of T residues in the nontranscribed strand of the DNA fixed to the paper) between the intensities of the 3' and 5' hybridized material in uninfected nuclei (Fig. 2A, lane 1), and that upon infection this attenuation is further enhanced (Fig. 2A, lane 2). The fragment corresponding to the 5' probe (−459 to +540) contains 221 T residues in the nontranscribed strand, of which only 110 are downstream to the promoter, whereas the 3' probe (+540 to +2448) contains 430 T residues. Therefore, a 5'/3' intensity ratio of 1/4 (110/430) would indicate equal amounts of RNA molecules (labeled at their U residues). In Fig. 2A, lane 1, however, the 5'/3' probe hybridization ratio is about 1/2, meaning a twofold excess of RNA hybridized to the 5' probe relative to that which hybridized to the 3' probe. In the blot of the *c-myc* gene, even though the 5'/3' probe intensity ratio is 2/1, when normalized to the number of T residues in the two probes, the calculated relative amounts of the RNA hybridized are identical, indicating that there is no obvious attenuation at the *c-myc* gene in the EA cells. We have previously shown by the same approach that transcription from the P4 promoter of MVM is attenuated (30). The block to transcriptional elongation at the *c-fos* attenuator may be modified by the addition of the known general transcriptional elongation factors, TFIIS and TFIIF (3), in a manner similar to that in which these factors affect the MVM attenuator (20). Furthermore, the *myc* attenuation signal seems to respond differently from *c-fos* and MVM attenuators to elongation factors and may in fact be dependent on an attenuation activity (29).

We therefore wished to investigate the possibility that upon viral infection, there is a reduction in the level of the general elongation factors. This putative reduction in elongation activity may account for the observed block to transcriptional elongation at the *c-fos* attenuator (this communication) as well as at the MVM attenuator, as seen in MVM-infected cells (19, 30). To address this question, we have partially purified transcription elongation complexes and added different cell extracts to them at elongation as described by Bengal et al. (5) and Krauskopf et al. (19). In this experiment, HeLa whole-cell extract (WCE) (prepared as described in reference 23) was incubated for 30 min at 30°C with ~8 µg of Ad2.fos (24) DNA (described in references 5 and 19) and transcription was carried out for a pulse-labeling of 1 min at 30°C (initiation step). The reaction mixture was applied onto a Sephacryl S-1000 mini column. Fractions containing the radioactive material were collected. Transcription elongation was then allowed to proceed for the indicated times at 30°C. Elongation of the complexes was performed in the presence of extracts from infected or uninfected EA cells or in the absence of extract, as indicated. Transcription was stopped and RNA was extracted and analyzed as described elsewhere (5, 19). As can be seen in Fig. 3A, in the absence of extract (lane 1), about 50% of the RNA was attenuated and the rest was full-length (for a schematic representation of the DNA template of Ad2.fos and the expected size of full-length run-off and attenuated RNAs, see panel C). When extracts from uninfected EA cells were added at the elongation stage (Fig. 3A, lane 2), the attenuated RNA was reduced by 60% and a corresponding increase in the amount of full-length RNA was observed. This effect was dependent on the amount of extract added at the elongation stage and was highly reproducible (data not shown). In contrast, when extracts from MVM-infected cells were added at the elongation step (Fig. 3A, lane

FIG. 3. Relief of the block to transcription elongation at the *c-fos* attenuator by addition of extracts from uninfected, but not from infected, EA cells. (A) Briefly initiated transcription complexes passed through a Sephacryl S-1000 column as described in the text were elongated in the absence of extract (C) (lane 1) or in the presence of 5 µl (25 µg) of WCE from uninfected (U) (lane 2) or infected (I) (lane 3) EA cells. M, pBR322 *MspI* DNA size markers (in bases). Runoff and attenuator RNAs are indicated by thick and thin arrows, respectively. (B) Aliquots were taken at the indicated time points during elongation in the absence of extract (C) (lanes 1 to 3) or in the presence of 10 µl (50 µg) of WCE from uninfected (U) (lanes 4 to 6) or infected (I) (lanes 7 to 9) EA cells. Runoff and attenuator RNAs are indicated by thick and thin arrows, respectively. M, DNA size markers as described in the legend to panel A. (C) Schematic representation of the template DNA of Ad2.fos, with the expected sizes of runoff and attenuated (Att.) RNAs indicated.

3), no shift toward the full-length RNA was observed. Kinetics of elongation are shown in Fig. 3B. In this experiment, samples were taken at different time points after the addition of the extracts. It can be seen that the rate of transcription elongation, as monitored by the time it took for the full-length transcript to appear, was increased when either extract was added (Fig. 3B, lanes 4 to 6 and 7 to 9) compared with that in the untreated reaction mixture (lanes 1 to 3). However, the ratio between full-length and attenuated transcripts in each reaction was not kinetically altered.

Taken together, these results suggest that at least two distinct elongation activities are present in WCEs from EA cells. One, which enhances the rate of transcription (similarly to the activity of TFIIF on the *c-fos* attenuator [3]), is unaffected by the infection. The other, which is able to specifically read through an attenuation site (similar to TFIIS [3]), is inactivated following MVM infection of EA cells. This inactivation results in the inability of extracts from infected cells to support readthrough of transcription at the *c-fos* attenuator (this paper), as well as the MVM and Ad2 attenuators (19). We propose that a similar mechanism accounts for the general shift in mRNA length observed upon infection.

The general decrease in transcript length resembles the phenomenon observed when cells are treated with the drug 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). DRB increases the early termination, 100 to 300 nt from sites of

initiation, of cellular heterologous nuclear RNA (hnRNA) synthesized by RNA polymerase II (33). Specifically, in the case of β -globin hnRNA synthesized in isolated nuclei from Friend erythroleukemic cells, it was shown that hnRNA from DRB-treated cells has a size of 9S or less while that of control cells contained an additional species corresponding to the 15S β -globin mRNA precursor (36). Examination of adenovirus (9) and simian virus 40 (21) transcriptional units has revealed that DRB causes early termination of RNA synthesis, resulting in accumulation of short RNA chains transcribed from promoter-proximal regions of the viral genome. The mechanism of DRB action is unknown.

Several models are consistent with the reduction in elongation activity induced by MVM infection. The first is that upon infection with MVM, there is a reduction in the production of a general transcription elongation protein. This reduction leads to a block of elongation at specific sites in genes that are modulated at the level of transcription elongation by the general elongation factors, such as *c-fos* and MVM P4. However, genes that are modulated through a distinct mechanism at the level of elongation, such as *c-myc*, are not similarly affected by the viral infection. The NS1 protein of MVM was shown to inhibit transcription from heterologous promoters and therefore may inhibit the transcription of a cellular elongation factor. We have checked by Western blotting (immunoblotting) of total cellular proteins whether there is a change in protein concentrations of the two identified elongation factors, TFIIS and TFIIIF, in infected versus uninfected cells and found that there is no significant difference (data not shown).

A second model which may be suggested is that an active mechanism of attenuation is induced upon viral infection. In this case, one of the viral proteins may act as an attenuation factor or may induce the activity of an attenuation factor. We are not inclined to favor this possibility, since in mixing experiments in which extracts from infected and uninfected cells were added together to partially purified transcription complexes, the effect was merely one of dilution and not a dominant one, as would have been expected for an active attenuation factor (data not shown).

A third model may be suggested, by which viral infection depletes an elongation factor posttranslationally without affecting its cellular concentration, for example, by sequestration, or it may induce a more subtle modification which renders the elongation factor inactive. For example, the viral gene NS1, which was suggested to act as an acidic transcriptional regulator (22), may possibly interact with an elongation factor and functionally deplete it from the infected cells by binding to it or modifying it. Furthermore, the fact that those attenuators that were affected by the infection, the MVM, Ad2, and *c-fos* attenuators, are responsive to the general elongation factors, in contrast to the *c-myc* attenuator, supports the elongation factor depletion model. This may indicate that a yet-unknown elongation factor is responsible for the decreased elongation activity or that a more subtle modification of a known factor is responsible for the observed effect.

This work was supported by grants from the National Institutes of Health (grant CA 14995); the United States-Israel Binational Science Foundation (BSF); the Israeli Academy of Sciences and Humanities; the ICRF; the Minerva Foundation, Munich, Germany; and the Leo Forchheimer Center for Molecular Genetics.

REFERENCES

- Aloni, Y., and N. Hay. 1985. Attenuation may regulate gene expression in animal viruses and cells. *Crit. Rev. Biochem.* **18**:327-383.
- Ben-Asher, E., and Y. Aloni. 1984. Transcription of minute virus of mice, an autonomous parvovirus, may be regulated by attenuation. *J. Virol.* **52**:266-276.
- Ben-Asher, E., and Y. Aloni. Unpublished data.
- Bender, T. P., C. B. Thomas, and W. M. Kuehl. 1987. Differential expression of *c-myc* mRNA in murine B lymphomas by a block to transcription elongation. *Science* **237**:1473-1476.
- Bengal, E., A. Goldring, and Y. Aloni. 1989. Transcription complexes synthesizing attenuated RNA can serve as a model system for analyzing elongation factors. *J. Biol. Chem.* **264**:18926-18932.
- Bentley, D. L., and M. Groudine. 1986. A block to elongation is largely responsible for decreased transcription of *c-myc* in differentiated HL60 cells. *Nature (London)* **321**:702-706.
- Bentley, D. L., and M. Groudine. 1988. Sequence requirements for premature termination of transcription in the human *c-myc* gene. *Cell* **53**:245-256.
- Berns, K. I., and M. A. Labow. 1987. Parvovirus gene regulation. *J. Gen. Virol.* **68**:601-614.
- Chodosh, L. A., A. Fire, M. Samuels, and P. A. Sharp. 1989. 5,6-Dichloro-1- β -D-ribofuranosylbenzimidazole inhibits transcription elongation by RNA polymerase II in vitro. *J. Biol. Chem.* **264**:2250-2257.
- Clemens, K. E., and D. J. Pintel. 1988. The two transcription units of the autonomous parvovirus minute virus of mice are transcribed in a temporal order. *J. Virol.* **62**:1448-1451.
- Cotmore, S. F., L. J. Sturzenbecker, and P. Tattersall. 1983. The autonomous parvovirus MVM encodes two nonstructural proteins in addition to its capsid polypeptides. *Virology* **129**:333-343.
- Cotmore, S. F., and P. Tattersall. 1986. Organization of nonstructural genes of the autonomous parvovirus minute virus of mice. *J. Virol.* **58**:724-732.
- Fort, P., J. Rech, A. Vie, M. Piechaczyk, J. M. Bonniieu, P. Jeanteur, and J. M. Blanchard. 1987. Regulation of *c-fos* gene expression in hamster fibroblasts: initiation and elongation of transcription and mRNA degradation. *Nucleic Acids Res.* **15**:5657-5667.
- Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the *c-fos* proto-oncogene. *Nature (London)* **311**:433-438.
- Hay, N., H. Skolnik-David, and Y. Aloni. 1982. Attenuation in the control of SV40 gene expression. *Cell* **29**:183-193.
- Kao, S.-Y., A. F. Calman, P. A. Luciw, and B. M. Peterlin. 1987. Antitermination of transcription within the long terminal repeat of HIV-1 by *tat* gene product. *Nature (London)* **330**:489-493.
- Kerppola, T. K., and C. M. Kane. 1988. Intrinsic sites of transcription termination and pausing in the *c-myc* gene. *Mol. Cell. Biol.* **8**:4389-4394.
- Kessler, M., and Y. Aloni. 1989. The block of transcription elongation at the SV40 attenuation site is decreased in vitro by oligomers complementary to segments of the attenuator RNA. *Gene* **84**:65-72.
- Krauskopf, A., E. Bengal, and Y. Aloni. 1991. The block to transcription elongation at the minute virus of mice attenuator is regulated by cellular elongation factors. *Mol. Cell. Biol.* **11**:3515-3521.
- Krauskopf, A., O. Resnekov, 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.
- Laub, O., E. B. Jakobovits, and Y. Aloni. 1980. 5,6-Dichloro-1- β -D-ribofuranosylbenzimidazole (DRB) enhances premature termination of late SV40 transcription. *Proc. Natl. Acad. Sci. USA* **77**:3297-3301.
- Legendre, D., and J. Rommelaere. 1992. Terminal regions of the NS-1 protein of parvovirus minute virus of mice are involved in cytotoxicity and promoter *trans* activation. *J. Virol.* **66**:5705-5713.
- Manley, J. L., A. Fire, A. Cano, A. Sharp, and M. Geffer. 1980. DNA-dependent transcription of adenovirus genes in a soluble whole cell extract. *Proc. Natl. Acad. Sci. USA* **77**:3855-3859.
- Mechti, N., M. Piechaczyk, J.-M. Blanchard, P. Jeanteur, and B. Lebleu. 1991. Sequence requirements for premature transcription arrest within the first intron of the mouse *c-fos* gene. *Mol. Cell. Biol.* **11**:2832-2841.

25. **Mok, M., A. Maderious, and S. Chen-Kiang.** 1984. Premature termination by human RNA polymerase II occurs temporally in the adenovirus major late transcriptional unit. *Mol. Cell. Biol.* **4**:2031–2040.
26. **Nepveu, A., and K. B. Marcu.** 1986. Intragenic pausing and antisense transcription within the murine c-myc locus. *EMBO J.* **5**:2859–2865.
27. **Piechaczyk, M., J. M. Blanchard, L. Marty, C. Dani, F. Panabiers, S. El Sabouty, P. Fort, and P. Jeanteur.** 1984. Post-transcriptional regulation of glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. *Nucleic Acids Res.* **12**:6951–6963.
28. **Pintel, D., D. Dadachanji, C. R. Astell, and D. C. Ward.** 1983. The genome of minute virus of mice, an autonomous parvovirus, encodes two overlapping transcription units. *Nucleic Acids Res.* **11**:1019–1038.
29. **Ragimov, N., E. Ben-Asher, and Y. Aloni.** Unpublished data.
30. **Resnekov, O., and Y. Aloni.** 1989. RNA polymerase II is capable of pausing and prematurely terminating transcription at a precise location both in vivo and in vitro. *Proc. Natl. Acad. Sci. USA* **86**:12–16.
31. **Resnekov, O., M. Kessler, and Y. Aloni.** 1989. RNA secondary structure is an integral part of the in vitro mechanism of attenuation in simian virus 40. *J. Biol. Chem.* **264**:9953–9959.
32. **Seiberg, M., M. Kessler, A. S. Levine, and Y. Aloni.** 1987. Human RNA polymerase II prematurely terminates transcription of adenovirus type 2 late transcription unit at a precise site that resembles a procaryotic termination signal. *Virus Genes* **1**:97–106.
33. **Tamm, I., and T. Kikuchi.** 1979. Early termination of heterologous nuclear RNA transcripts in mammalian cells: accentuation by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB). *Proc. Natl. Acad. Sci. USA* **76**:5750–5754.
34. **Tattersall, P.** 1972. Replication of the parvovirus MVM. I. Dependence of virus multiplication and plaque formation on cell growth. *J. Virol.* **10**:586–590.
35. **Toohy, M. G., and K. A. Jones.** 1989. In vitro formation of short RNA polymerase II transcripts that terminate within the HIV-1 and HIV-2 promoter-proximal downstream regions. *Genes Dev.* **3**:265–282.
36. **Tweeten, K. A., and G. R. Molloy.** 1981. Induction of premature termination of transcription of the mouse β -globin gene by 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB). *Nucleic Acids Res.* **9**:3307–3319.
37. **Yanofsky, C.** 1988. Transcription attenuation. *J. Biol. Chem.* **263**:609–612.