In Vitro Transcription of a Cloned Vaccinia Virus Gene by a Soluble Extract Prepared from Vaccinia Virus-Infected HeLa Cells

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Faithful transcription of a vaccinia virus gene was accomplished in vitro by using a soluble extract prepared from vaccinia virus-infected HeLa cells. Specific transcription of the cloned vaccinia virus gene was detected by using template DNA restricted within the transcribed region. The vaccinia virus gene was not transcribed by extracts prepared from uninfected HeLa cells even with supplementation by purified vaccinia virus RNA polymerase, nor was a clone of adenovirus 2 DNA bearing the major late promoter transcribed by the extract from vaccinia virus-infected HeLa cells. Thus, infection by vaccinia virus altered cellular transcriptional specificity to favor expression of vaccinia virus genes. RNA synthesis by the infected cell extract was resistant to α -amanitin but strongly inhibited by β , γ -imido ATP and novobiocin.

Vaccinia virus has been a very useful model system for analysis of eucaryotic transcription. Vaccinia virion cores transcribe early viral genes in vitro (13) and thus contain all of the enzymes required for early transcription. A number of enzymes have been isolated from viral core particles and have been characterized. Of these, the DNA-dependent RNA polymerase (2, 32), guanylyltransferase and 7-methyltransferase (18, 21), 2'-O-methyltransferase (1), and polyadenylate polymerase (6, 22) are clearly required for transcription. Several other virion enzymes which may function in transcription have been described, including 5'-phosphate polyribonucleotide kinase (31), protein kinase (14), two distinct nucleic acid-dependent ribonucleoside triphosphate phosphohydrolases (25), a single-strand-specific nuclease(s) (26, 28), endoribonuclease (24), two abundant DNA-binding proteins (3, 11), and a type I DNA topoisomerase (3). Purified preparations of vaccinia virus RNA polymerase are similar to cellular RNA polymerase II isolates in their requirement for Mn^{2+} and single-stranded DNA in vitro and their inability to transcribe duplex DNA templates. Thus, other proteins appear to be required for proper initiation of transcription from native DNA templates (19). Furthermore, RNA synthesis from intact vaccinia virus core particles but not by purified vaccinia virus RNA polymerase is sensitive to β , γ -imido ATP (AMP-PNP) and also to novobiocin (7, 8, 30). Thus, vaccinia virus is an ideal model system for analysis of the factors required for initiation of transcription.

Recently, the development of soluble cellular extracts capable of faithful initiation of transcription in vitro has provided a powerful method for analysis of transcriptional initiation events (16, 35). Defined duplex DNA templates have been transcribed in vitro to yield truncated transcripts characteristic of initiation from a given promoter. Cloning and RNA mapping of the vaccinia virus genome (10) have made possible analysis of transcription of defined vaccinia virus genes in vitro. In this paper I describe the development and characterization of a soluble extract from vaccinia virus-infected HeLa cells which faithfully initiates transcription of a cloned vaccinia virus gene in vitro.

MATERIALS AND METHODS

Materials. Unlabeled nucleotides and novobiocin were obtained from Sigma Chemical Co. AMP-PNP was purchased from Boehringer Mannheim Biochemicals. [α - ^{32}P]GTP was purchased from Amersham Corp.

Preparation of nucleic acids. Plasmid pSmaI-F bearing adenovirus type 2 (Ad2) SmaI fragment F (11.6 to 18.2 map units) was provided by H. Furneaux. Plasmid pAG4 (34) bearing a 960-base pair (bp) insertion of vaccinia virus DNA was provided by B. Moss. Supercoiled plasmid DNAs were isolated as described previously (20). DNA products of restriction endonuclease digests were analyzed on 4% polyacrylamide gels under conditions described previously (3).

Virus. Vaccinia virus strain WR was purified from infected HeLa cells through two sucrose gradients, as described previously (9).

Preparation of extracts from vaccinia virus-infected HeLa cells. HeLa cells at a density of 5×10^5 cells per ml were infected with 280 PFU of vaccinia virus per cell. At 1 and 5 h after infection extracts were prepared as described previously (16). Extracts were stored at -70°C in 200- μl portions.

Preparation of vaccinia virus DNA-dependent RNA polymerase. Vaccinia virus RNA polymerase was purified from virions through phosphocellulose and was assayed as described previously (32).

Protein determination. Protein concentrations were determined by using the method of Bradford (5).

Assay for in vitro transcription. The standard reaction mixture (50 μl) contained 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.9), 5 mM $MgCl_2$, 2 mM dithiothreitol, 1 mM ATP, 1 mM CTP, 1 mM UTP, 0.2 mM [α - ^{32}P]GTP (20,000 to 40,000 cpm/pmol), 1.0 μg of DNA, and 20 μl of extract. The assay mixtures were incubated at 30°C for 60 min, and reactions were terminated by adding 1 μl of 1 mM GTP and 150 μl of 4 M urea-1.0% sodium dodecyl sulfate-20 mM EDTA. The RNA was extracted with 200 μl of phenol-chloroform (1:1), and the organic phase was extracted with a solution containing 7 M urea, 0.35 M NaCl, 10 mM Tris-hydrochloride (pH 8.0), 2 mM EDTA, and 0.5% sodium dodecyl sulfate. The aqueous phases were pooled, extracted again, and precipitated with ethanol by using 50 μg of *Escherichia coli* tRNA as a carrier. The pellets were suspended in 300 μl of 1 M ammonium

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FIG. 1. Restriction sites and truncated transcripts of the pAG4 insertion region. A 960-bp *SalI* fragment of the vaccinia virus terminal repeat was ligated into the *SalI* site of pBR322 DNA (34). The promoter of the 7,500-dalton gene is indicated by the open triangle. Transcription of pAG4 restricted with *DdeI*, *SalI*, and *BglI* yielded transcripts of 450, 730, and 1,010 nucleotides, respectively.

acetate and precipitated with ethanol. Then the pellets were suspended in 300 μ l of 0.3 M sodium acetate and precipitated with ethanol. The final pellets were suspended in 15 μ l of a solution containing 50 mM boric acid, 5 mM sodium borate, 10 mM sodium sulfate, and 0.2 mM EDTA (BBE) supplemented with 2 μ l of 0.2 M methylmercury hydroxide and 4 μ l of a solution containing 50% sucrose, 0.02% xylene cyanol, and 0.02% bromophenol blue. Samples were electrophoresed on 4% polyacrylamide gels in BBE containing 10 mM methylmercury hydroxide at 80 V for 3 h. Autoradiograms of the dried gels were prepared by using Kodak XAR-5 film.

RESULTS

Restricted DNA templates for in vitro transcription. In vitro studies of specific transcriptional events can be performed by using DNA-dependent cell extracts and template DNA restricted within the coding sequence so that truncated transcripts of defined length are synthesized. The template used in this study, plasmid pAG4, contains a 960-bp *SalI* fragment isolated from the vaccinia virus inverted terminal repeat and cloned into the unique *SalI* site of pBR322 (34). The insertion contains the promoter and 730 bp of the coding sequence of a gene encoding an early protein with an M_r of 7,500, as well as about 230 bp of sequences that are 5' proximal to the site of transcriptional initiation. A diagram indicating the relevant restriction sites in the region of the pAG4 insertion is shown in Fig. 1. Restriction of pAG4 with *SalI* releases the insertion, which, if transcribed, would result in a truncated 730-nucleotide transcript that could be readily detected by denaturing polyacrylamide gel electrophoresis. Therefore, the *SalI* digest of pAG4 was routinely used as a template for in vitro transcription studies. I deduced from the *BamHI-DdeI* double digest of pAG4 (data not shown) that the orientation of the vaccinia virus DNA insertion within pAG4 was such that the direction of transcription from the 7,500-dalton gene promoter was away from the unique *BamHI* site of the vector and toward the *BglI* site, as shown in Fig. 1. Therefore, restriction of pAG4 with *DdeI* should have yielded a shortened transcript of about 450 nucleotides, and similarly, restriction with *BglI* should have produced a transcript of about 1,010 nucleotides (Fig. 1). RNA products of these predicted sizes were observed when these DNAs were transcribed in extracts of vaccinia virus-infected HeLa cells as described above (data not shown). These RNAs were not observed as products of in vitro transcription assays programmed with the corresponding restricted pBR322 DNAs. These results support the independently reported conclusion (27) that RNA synthesis initiates at the same site on this region of vaccinia virus DNA in vivo and in vitro in extracts of vaccinia

virus-infected HeLa cells. The *SmaI* digest of a plasmid bearing Ad2 *SmaI* fragment F containing the major late promoter was used as the template for in vitro transcription by uninfected HeLa extracts to yield a 560-nucleotide transcript (35).

Comparison of uninfected and vaccinia virus-infected HeLa cell extracts for transcription of adenovirus and vaccinia virus genes. Extracts were prepared from uninfected HeLa cells and also from HeLa cells infected with 280 PFU of vaccinia virus per cell at 1 and 5 h after infection, as described above. The protein concentrations of these extracts were determined to be about 8 mg/ml. The transcription of adenovirus *SmaI* fragment F and *SalI*-digested pAG4 was determined for all three extracts as described above (Fig. 2). Assay mixtures lacking an added DNA template (Fig. 2, lanes A) showed no RNA synthesis, demonstrating the DNA dependence of transcription by these extracts. Other assay mixtures (lanes B) were programmed with Ad2 *SmaI*-F DNA and showed the expected RNA product of 560 nucleotides for the uninfected extract. This band was greatly reduced in the assay mixture containing the 1-h-infected cell extract (VH1 extract) and completely absent in the assay mixture containing the 5-h-infected cell extract (VH5 extract), indicating that vaccinia virus infection eliminated RNA polymerase II transcription of *SmaI* fragment F. In other assays (lanes C) the extracts were programmed with the *SalI* digest of pAG4. The 730-nucleotide RNA product was observed clearly only with the VH5 extract, indicating that the vaccinia virus gene can be transcribed most efficiently by extracts from infected cells prepared several hours after infection. These results are consistent with those reported by other workers (27). The higher-molecular-weight bands (especially the 960-nucleotide band seen clearly in HeLa cell lane C) evident in all lanes programmed with exogenous DNA were determined to be sensitive to DNase (data not shown). Other investigators have also considered such bands to be artifacts of end labeling by crude lysates (17). The 560- and 730-nucleotide RNAs were resistant to DNase treatment.

Optimal reaction conditions for vaccinia virus transcription in vitro. Optimal synthesis of the 730-nucleotide RNA required the addition of 20 μ l of extract (8 mg of protein per ml) and 1.5 μ g of DNA per 50- μ l assay (data not shown).

FIG. 2. In vitro transcription by uninfected and vaccinia virus-infected HeLa cell extracts. Products of transcription by uninfected (HeLa), VH1, and VH5 cell extracts were programmed with no DNA (lanes A), *SmaI*-digested p*SmaI*-F (lanes B), and *SalI*-digested pAG4 (lanes C). Products that were 560, 730, and 960 nucleotides long are indicated. Lane M contained *HaeIII*-digested ϕ X174 DNA that was 5'-labeled with [γ - 32 P]ATP and polynucleotide kinase (used as size markers).

Higher concentrations of extract or of DNA resulted in synthesis of spurious products. Similar sharp optima for extract and DNA concentrations are characteristic of related in vitro transcription systems (16). Synthesis of the 730-nucleotide RNA required relatively high concentrations (1 mM) of ribonucleoside triphosphates, perhaps because of the presence of vaccinia virus phosphohydrolases in the infected cell extracts. Specific RNA synthesis occurred at Mg^{2+} concentrations of 5 to 10 mM, conditions similar to the conditions required for transcription of DNA within intact vaccinia virus cores. However, addition of 5 to 10 mM Mn^{2+} to assay mixtures containing 5 mM Mg^{2+} strongly inhibited synthesis of the 730-nucleotide transcript (data not shown), whereas Mn^{2+} was required for activity of the RNA polymerase purified from vaccinia virions. Thus, the soluble infected cell extract retained an important characteristic of viral RNA synthesis in vivo which was lost upon purification of the vaccinia virion RNA polymerase.

α -Amanitin resistance of in vitro transcription by vaccinia virus-infected HeLa cell extracts. α -Amanitin, a potent inhibitor of RNA polymerase II, has been shown to completely inhibit in vitro transcription of Ad2 *Sma*I fragment F by uninfected HeLa cell extracts (35). The α -amanitin sensitivity of transcription by the VH5 extract was examined. Transcription of Ad2 *Sma*I-F and transcription of *Sall*-digested pAG4 were determined as described above for the HeLa cell and VH5 extracts, respectively, in the presence and absence of 1 μ g of α -amanitin per ml (Fig. 3). Synthesis of the 560-nucleotide RNA was completely abolished by α -amanitin (Fig. 3, HeLa cell extract, lane C). However, synthesis of the 730-nucleotide transcript by the VH5 extract was completely resistant to 1 μ g of α -amanitin per ml (Fig. 3, VH5 extract, lane C), indicating that RNA polymerase II does not catalyze the synthesis of this transcript. This RNA product is synthesized by the α -amanitin-resistant vaccinia virus RNA polymerase since its synthesis by infected cell extracts is inhibited by antibody directed against purified vaccinia virus RNA polymerase (27).

Supplementation of uninfected HeLa cell extracts with vaccinia virus RNA polymerase. Both vaccinia virus RNA

FIG. 3. α -Amanitin sensitivity of transcription by uninfected and vaccinia virus-infected HeLa extracts. Uninfected (HeLa) and VH5 cell extracts were assayed for transcription of p*Sma*I-F and *Sall*-digested pAG4, respectively, in the presence and absence of 1 μ g of α -amanitin per ml. Lanes A, no DNA; lanes B, DNA; lanes C, DNA plus 1 μ g of α -amanitin per ml.

FIG. 4. Supplementation of extracts with vaccinia virus RNA polymerase. Uninfected and vaccinia virus-infected HeLa cell extracts were assayed for transcription of *Sall*-digested pAG4 with or without supplementation with purified vaccinia virus RNA polymerase. Uninfected extract assay mixtures contained no DNA (lane A), *Sma*I-F DNA (lane B), and *Sall*-digested pAG4 (lanes C, D, and E). VH5 extract assay mixtures contained no DNA (lane F) or *Sall*-digested pAG4 (lanes G, H, and I). Lanes D and H contained assay mixtures supplemented with 0.02 U of vaccinia virus RNA polymerase, and lanes E and I contained assay mixtures supplemented with 0.1 U of RNA polymerase. Lanes J and K contained assay mixtures of *Sall*-digested pAG4 transcription by 0.02 and 0.1 U of vaccinia virus RNA polymerase, respectively.

polymerase and RNA polymerase II require additional factors to utilize duplex DNA as a template. Therefore, it was of interest to determine whether those factors present in extracts of uninfected cells could substitute for the factors present in infected cells or whether there were virus-specific proteins other than the RNA polymerase which were required for in vitro transcription. Assays were performed as described above, using both HeLa cell and VH5 extracts with *Sall*-digested pAG4 as the template with or without supplementation with 0.02 or 0.1 U of purified vaccinia virus RNA polymerase (Fig. 4). Supplementation with vaccinia virus RNA polymerase did not result in detectable synthesis of the 730-nucleotide transcript by the HeLa cell extract (Fig. 4, lanes D and E), nor did it significantly alter the transcription of infected cell extracts (lanes H and I). RNA polymerase alone did not synthesize any detectable 730-nucleotide RNA (lanes J and K). Therefore, in vitro transcription of pAG4 requires virus-specific factors other than vaccinia virus RNA polymerase.

Sensitivity of transcription by HeLa cell and VH5 extracts to AMP-PNP and novobiocin. Transcription by vaccinia virus core particles requires hydrolysis of ATP to ADP and P_i and therefore is inhibited by ATP analogs, such as AMP-PNP, in which hydrolysis of the β,γ phosphate bond is restricted (8, 30). This analog inhibits the vaccinia virus ribonucleoside triphosphate phosphohydrolases but not the vaccinia virus RNA polymerase (30). Novobiocin, an inhibitor of the vaccinia virus DNA topoisomerase, also inhibits transcription by intact vaccinia virus cores (7). Therefore, the sensitivity of in vitro transcription to these compounds was determined. Assays of the VH5 extract with *Sall*-digested pAG4 were performed as described above in the presence and absence of 1 mM AMP-PNP or novobiocin. The assay with the ATP analog was performed in the absence of ATP. The results are shown in Fig. 5. Transcription of the 730-nucleotide RNA by the VH5 extract was strongly inhibited

FIG. 5. Transcription of *Sall*-digested pAG4 by vaccinia virus-infected HeLa extracts in the presence and absence of AMP-PNP and novobiocin. Lane A contained an assay mixture programmed with no DNA, and lane B contained the 730-nucleotide transcript synthesized from *Sall*-digested pAG4. Lane C shows the results of an assay performed in the absence of ATP and in the presence of 1 mM AMP-PNP. Lane D shows the results of an assay performed in the presence of 1 mM novobiocin.

by 1 mM AMP-PNP (Fig. 5, lane C). This result is consistent with a requirement for vaccinia virus phosphohydrolase I or II activity for in vitro transcription in this system. Complete inhibition of transcription by 1 mM novobiocin was observed (lane D), indicating a possible requirement for DNA topoisomerase I activity for in vitro transcription. Synthesis of the 560-nucleotide RNA by uninfected HeLa cell extracts programmed with Ad2 *Sma*I fragment F DNA was also completely inhibited by 1 mM novobiocin (data not shown).

DISCUSSION

Significant insights into the molecular mechanisms of eucaryotic transcription have been obtained from studies of vaccinia virus transcription, notably the discovery of polyadenylation (12) and the characterization of the mechanism of RNA capping (29). Since vaccinia virus encapsidates the enzymes required for early gene expression, the virion core particles have been used to analyze transcription in vitro. However, understanding the precise mechanisms of transcription required the development of a soluble system capable of faithful transcription in vitro. Cloning and mRNA mapping of the vaccinia virus genome have provided numerous potential templates for transcriptional analyses (10). Plasmid pAG4 was selected for these studies since it contains an early vaccinia virus gene which has been mapped precisely (34). The promoter of this gene has been shown to function in vivo (15), and the DNA sequence of the 5'-proximal region has been determined (33). Transcription of *Sall*-digested pAG4 results in a 730-nucleotide transcript which can be readily detected by denaturing gel electrophoresis.

Extracts for transcriptional assays were prepared from vaccinia virus-infected HeLa cells by a procedure which yields transcriptionally active extracts from uninfected HeLa

cells (16). The infected cell extract synthesized the expected 730-nucleotide product which initiates at the same site as the in vivo transcript, as shown by other workers (27). Interestingly, uninfected HeLa cell extracts fail to transcribe the vaccinia virus gene, and conversely vaccinia virus-infected HeLa cell extracts do not transcribe the adenovirus clone bearing the RNA polymerase II major late promoter. Thus, a virus-specific function(s) is required for vaccinia virus transcription, and infection by vaccinia virus inactivates RNA polymerase II transcription. Greatly reduced levels of cellular mRNA have been reported for vaccinia virus-infected cells (4). Synthesis of RNA by infected cell extracts is resistant to α -amanitin (27), as is RNA synthesized by purified vaccinia virus RNA polymerase (2, 32). The vaccinia virus RNA polymerase is required for synthesis of the 730-nucleotide RNA since antibody directed against it eliminates pAG4 transcription (27).

Supplementation of uninfected extracts by purified vaccinia virus RNA polymerase did not result in any detectable synthesis of the 730-nucleotide transcript. Thus, a virus-specific factor(s) other than RNA polymerase is required for specific transcription in vitro. Inhibition of transcription by the VH5 extract by AMP-PNP indicates a requirement for ATP hydrolysis to ADP and P_i , as has been observed for transcription in vaccinia virus core particles (8, 30). Therefore, phosphohydrolase I or II may be required for transcription by the infected cell extract. AMP-PNP also inhibits the vaccinia virus topoisomerase (7) but at concentrations higher than 1 mM, which was used in these studies. Novobiocin, a potent inhibitor of the topoisomerase, completely inhibits transcription by both infected and uninfected HeLa extracts, suggesting a requirement for topoisomerase activity for in vitro transcription. It should be noted that novobiocin may not be specific for the topoisomerase, but it does not inhibit vaccinia virus RNA polymerase or phosphohydrolases I and II (D. Foglesong and R. Guggenheimer, unpublished data). The apparent requirement for topoisomerase activity is surprising since the templates used were linear DNAs which are not subject to any topological constraint. However, replication of linear duplex adenovirus DNA in vitro also requires a type I topoisomerase (23). Thus, topoisomerase activity may facilitate the movement of transcription and replication forks through duplex DNA.

Since vaccinia virus encapsidates all of the enzymes required for early transcription, virion extracts were assayed for activity in transcribing pAG4 in vitro. Initial attempts in which I used extracts prepared by conventional methods (32) were unsuccessful even when the extracts were supplemented with purified vaccinia virus RNA polymerase. However, virion extracts concentrated by ammonium sulfate precipitation or negative pressure dialysis synthesized the 730-nucleotide product (unpublished data). Thus, high protein concentrations may be required for this in vitro system, as for other transcriptional systems (16). Virion extracts may be more useful than infected cell extracts for some transcription studies since the proteins required for transcription are of much greater specific activity in virion extracts than those of infected cell extracts. Other investigators have also transcribed cloned vaccinia virus genes in vitro by using virion extracts (B. Moss, personal communication; F. Golini and J. Kates, Int. Symp. Pox/Iridoviruses, Abstr. ThA6, presented 26 July 1984 at Madison, Wis.). The development of soluble in vitro systems for vaccinia virus transcription will greatly facilitate the analysis of the protein factors required for accurate initiation of transcription and also the DNA sequences required for recognition by those factors.

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LITERATURE CITED

- Barbosa, E., and B. Moss. 1977. mRNA (nucleoside-2'-)-methyltransferase from vaccinia virus. *J. Biol. Chem.* **253**:7692-7697.
- Baroudy, B. M., and B. Moss. 1980. Purification and characterization of a DNA-dependent RNA polymerase from vaccinia virions. *J. Biol. Chem.* **255**:4372-4380.
- Bauer, W. R., E. C. Resser, J. R. Kates, and J. N. Patzke. 1977. A DNA nicking-closing enzyme encapsidated in vaccinia virus: partial purification and properties. *Proc. Natl. Acad. Sci. U.S.A.* **74**:1841-1845.
- Boone, R. F., and B. Moss. 1978. Sequence complexity and relative abundance of vaccinia mRNAs synthesized in vivo and in vitro. *J. Virol.* **26**:554-569.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.
- Brakel, C., and J. Kates. 1974. Poly(A) polymerase from vaccinia virus-infected cells. *J. Virol.* **14**:715-723.
- Foglesong, P. D., and W. R. Bauer. 1984. Effects of ATP and inhibitory factors on the activity of vaccinia virus type I topoisomerase. *J. Virol.* **49**:1-8.
- Gershowitz, A., R. F. Boone, and B. Moss. 1980. Multiple roles for ATP in the synthesis and processing of mRNA by vaccinia virus: specific inhibitory effects of adenosine (β,γ -imido) triphosphate. *J. Virol.* **27**:399-408.
- Hruby, D. E., L. A. Guarino, and J. R. Kates. 1979. Vaccinia virus replication. I. Requirement for the host cell nucleus. *J. Virol.* **29**:705-715.
- Isle, H. B., L. Venkatesan, and B. Moss. 1981. Cell-free translation of early and late mRNAs selected by hybridization to cloned DNA fragments derived from the left 14 million to 72 million daltons of the vaccinia virus genome. *Virology* **112**:306-317.
- Kao, S., E. Resser, J. Kates, and W. Bauer. 1981. Purification and characterization of a superhelix binding protein from vaccinia virus. *Virology* **111**:500-508.
- Kates, J. 1970. Transcription of the vaccinia virus genome and the occurrence of polyriboadenylic acid sequences in messenger RNA. *Cold Spring Harbor Symp. Quant. Biol.* **35**:743-752.
- Kates, J. R., and B. R. McAuslan. 1967. Messenger RNA synthesis by a "coated" viral genome. *Proc. Natl. Acad. Sci. U.S.A.* **57**:314-320.
- Kleiman, J. H., and B. Moss. 1975. Characterization of a protein kinase and two phosphate acceptor proteins from vaccinia virions. *J. Biol. Chem.* **250**:2420-2429.
- Mackett, M., G. L. Smith, and B. Moss. 1982. Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proc. Natl. Acad. Sci. U.S.A.* **79**:7415-7417.
- Manley, J. L., A. Fire, A. Cano, P. A. Sharp, and M. L. Gelfer. 1980. DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. *Proc. Natl. Acad. Sci. U.S.A.* **77**:3855-3859.
- Manley, J. L., and M. L. Gelfer. 1981. Transcription of mammalian genes in vitro, p. 369-382. *In* G. U. Chirikjian and T. S. Papas (ed.), *Gene amplification and analysis*, vol. 2. Structural analysis of nucleic acid. Elsevier, New York.
- Martin, S. A., E. Paoletti, and B. Moss. 1975. Purification of mRNA guanylyltransferase and mRNA (guanine-7-)methyltransferase from vaccinia virions. *J. Biol. Chem.* **250**:9322-9329.
- Matsui, T., J. Segall, A. Weil, and R. G. Roeder. 1980. Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. *J. Biol. Chem.* **255**:11992-11996.
- Mickel, S., and W. Bauer. 1976. Isolation by tetracycline selection of small plasmids derived from R-factor R12 in *Escherichia coli* K-12. *J. Bacteriol.* **127**:644-655.
- Monroy, G., E. Spencer, and J. Hurwitz. 1978. Purification of mRNA guanylyltransferase from vaccinia virions. *J. Biol. Chem.* **253**:4481-4489.
- Moss, B., E. N. Rosenblum, and A. Gershowitz. 1975. Characterization of polyadenylate polymerase from vaccinia virions. *J. Biol. Chem.* **250**:4722-4729.
- Nagata, K., R. A. Guggenheimer, and J. Hurwitz. 1983. Adenovirus DNA replication in vitro: synthesis of full-length DNA with purified proteins. *Proc. Natl. Acad. Sci. U.S.A.* **80**:4266-4270.
- Paoletti, E., and B. R. Lipinskas. 1978. Soluble endoribonuclease activity from vaccinia virus: specific cleavage of virion-associated high-molecular-weight RNA. *J. Virol.* **26**:822-824.
- Paoletti, E., and B. Moss. 1974. Two nucleic acid-dependent nucleoside triphosphate phosphohydrolases from vaccinia virus: purification and characterization. *J. Biol. Chem.* **249**:3287-3291.
- Pogo, B. G. T., and M. T. O'Shea. 1977. Further characterization of deoxyribonucleases from vaccinia virus. *Virology* **77**:56-66.
- Puckett, C., and B. Moss. 1983. Selective transcription of vaccinia virus genes in template dependent soluble extracts of infected cells. *Cell* **35**:441-448.
- Rosemond-Hornbeak, H., E. Paoletti, and B. Moss. 1974. Single-stranded deoxyribonucleic acid-specific nuclease from vaccinia virus. *J. Biol. Chem.* **249**:3287-3291.
- Shuman, S., and J. Hurwitz. 1981. Mechanism of mRNA capping by vaccinia virus guanylyltransferase: characterization of an enzyme-guanylate intermediate. *Proc. Natl. Acad. Sci. U.S.A.* **78**:187-191.
- Shuman, S., E. Spencer, H. Furneaux, and J. Hurwitz. 1980. The role of ATP in in vitro vaccinia virus RNA synthesis. *J. Biol. Chem.* **255**:5396-5403.
- Spencer, E., D. Loring, J. Hurwitz, and G. Monroy. 1978. Enzymatic conversion of 5'-phosphate-terminated RNA to 5'-di- and triphosphate-terminated RNA. *Proc. Natl. Acad. Sci. U.S.A.* **75**:4793-4797.
- Spencer, E., S. Shuman, and J. Hurwitz. 1980. Purification and properties of vaccinia virus DNA-dependent RNA polymerase. *J. Biol. Chem.* **255**:5388-5395.
- Venkatesan, S., B. M. Baroudy, and B. Moss. 1981. Distinctive nucleotide sequences adjacent to multiple initiation and termination sites of an early vaccinia virus gene. *Cell* **125**:805-813.
- Venkatesan, S., and B. Moss. 1981. In vitro transcription of the inverted terminal repetition of the vaccinia virus genome: correspondence of initiation and cap sites. *J. Virol.* **37**:738-747.
- Weil, P. A., D. S. Luse, J. Segall, and R. G. Roeder. 1979. Selective and accurate initiation of transcription at the Ad2 major late promoter in a soluble system dependent on purified RNA polymerase II and DNA. *Cell* **18**:469-484.