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Site-specific RNA cleavage generates the 3′ end of a poxvirus late mRNA

(RNA processing/RNA 3′ end formation/vaccinia virus/cowpox virus)

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ABSTRACT **The cowpox virus late mRNAs encoding the major protein of the A-type inclusions have 3′ ends corresponding to a single site in the DNA template. The DNA sequence of the *Alu* I–*Xba* I fragment at this position encodes an RNA cis-acting signal, designated the AX element, which directs this RNA 3′ end formation. In cells infected with vaccinia virus the AX element functions independently of either the nature of the promoter element or the RNA polymerase responsible for generating the primary RNA. At late times during virus replication, vaccinia virus induces or activates a site-specific endoribonuclease that cleaves primary RNAs within the AX element. The 3′ end produced by RNA cleavage is then polyadenylylated to form the 3′ end of the mature mRNA. Therefore, the poxviruses employ at least two mechanisms of RNA 3′ end formation during the viral replication cycle. One mechanism, which is operative at early times in viral replication, involves the termination of transcription [Rohrmann, G., Yuen, L. & Moss, B. (1986) *Cell* 46, 1029–1035]. A second mechanism, which is operative at late times during viral replication, involves the site-specific cleavage of primary RNAs.**

The 3′ ends of mRNAs are formed in eukaryotic cells either by the posttranscriptional processing of a primary RNA or, less commonly, by the termination of transcription (reviewed in refs. 1 and 2). The 3′ ends of the mRNAs of DNA viruses of most types are formed in a similar way, because most viruses employ the transcriptional apparatus of the cell to synthesize viral mRNAs.

The poxviruses differ from other DNA viruses in that they replicate in the cytoplasm of the cell, employing numerous viral enzymes, instead of host cell enzymes, to synthesize their RNAs. Poxviral proteins known to be involved in RNA synthesis and processing include a multisubunit RNA polymerase that resembles eukaryotic RNA polymerase II, several transcription factors, a capping-enzyme complex, an RNA methyltransferase, a poly(A) polymerase, and an endoribonuclease (reviewed in ref. 3). This assemblage of viral proteins suggests that the poxvirus may encode all the factors necessary for viral RNA synthesis, including those required for RNA 3′ end formation.

The transcription of poxvirus genes is a temporally regulated process. Early genes are transcribed before viral DNA replication, intermediate genes are transcribed after the onset of viral DNA replication, and late genes are transcribed after the expression of the intermediate genes (reviewed in ref. 4). The processes used to form the 3′ ends of viral RNAs are temporally regulated also. The 3′ ends of the early RNAs are generated by the termination of transcription, which occurs about 50 nucleotides downstream of the signal sequence 5′-UUUUUNU-3′ in the nascent RNA (5, 6). Interestingly, the process generating the termination of transcription of early genes does not appear to operate after the onset of viral DNA replication (7–9). RNA transcripts of most characterized late genes appear to be heterogeneous in length, lacking the defined 3′ ends characteristic of the early mRNAs (10, 11). However, a few late transcription units whose RNAs are homogeneous in length have been identified. These include the cowpox virus gene encoding the most abundant viral protein, the major protein component of the A-type inclusion (ATI) bodies (12, 13); the equivalent vaccinia virus gene (14, 15); and the telomeric transcription units of vaccinia virus, cowpox virus, and raccoonpox virus (16).

The mechanisms involved in the generation of the defined 3′ ends of late viral RNAs are the subject of this study. Here we demonstrate that the 3′ ends of the late RNAs encoding the ATI protein are generated not by the termination of transcription, as is the case for 3′ end formation of early RNAs, but by the site-specific cleavage of a precursor RNA.

MATERIALS AND METHODS

Viruses and Cells. Vaccinia virus (Western Reserve strain), vaccinia virus vTF7-3 (17), and vaccinia virus recombinants were cultured either in human 143 cells or HeLa S3 cells, as described (12, 18).

Construction of Vaccinia Virus A461. An 850-base-pair (bp) *Bam*HI fragment containing the coding region for the bacterial chloramphenicol acetyltransferase (CAT) gene was isolated from plasmid p863 (obtained from E. Linney, Duke University, Durham, NC), which is a derivative of pSV2-cat^S (19). This was inserted into the *Bam*HI site in pTF7-5 (20), generating plasmid p1373, in which the CAT gene was in the same orientation as the phage T7 promoter. A 2080-bp *Eco*RI fragment containing the region corresponding to the 3′ end of the late mRNAs encoding the major ATI protein was obtained from the *Kpn* I G-fragment of the DNA of cowpox virus (12). This fragment was inserted at the *Eco*RI site in the CAT gene in p1373, generating plasmid p2098, in which the viral thymidine kinase gene, the phage T7 promoter, the CAT gene, and the 3′ end of the gene encoding the ATI protein were all in the same orientation. A vaccinia virus recombinant, A461, containing this gene construction (Fig. 1) was generated by standard procedures (21), using plasmid p2098 as the insertion vector.

Preparation of Whole-Cell Extracts. Whole-cell extracts were prepared (22) from uninfected HeLa cells, HeLa cells infected with vaccinia virus, and mouse L cells infected with vaccinia virus. Cells were infected with 10 plaque-forming units of virus per cell. The protein concentration of each extract was adjusted to 3.25 mg/ml.

RNA Cleavage Assays. Unlabeled and ^{32}P-labeled RNA substrates were prepared by *in vitro* transcription (16) of the

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Abbreviations: ATI, A-type inclusion; CAT, chloramphenicol acetyltransferase.

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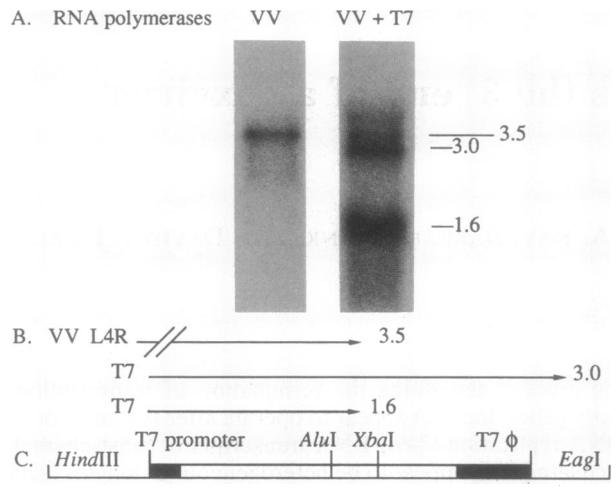

FIG. 1. Site-specific RNA 3′ end formation is not dependent upon either the promoter or the RNA polymerase. (*A*) Northern blot analysis of late RNAs synthesized in cells infected either with vaccinia virus A461 (VV) or with A461 and vaccinia virus vTF7-3, which encodes the phage T7 RNA polymerase (VV + T7). RNAs were extracted from cells 20 hr after infection, resolved by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde, and then transferred to a nylon membrane. Transcripts of the CAT gene were detected by DNA·RNA hybridization using a probe specific for CAT gene sequences. The sizes of the CAT transcripts are indicated in kilobases. (*B*) Map of the various CAT transcription units. Transcription is under the control of either the late promoter of the vaccinia virus L4R gene or the phage T7 promoter (RNA lengths are indicated in kilobases). Arrowheads indicate the positions of the RNA 3′ ends. (*C*) Composition of the portion of the vaccinia virus *Hin*dIII J-fragment containing the modified CAT gene inserted into the viral thymidine kinase gene. This construction is contained both in plasmid p2098 and in virus A461. It comprises the phage T7 promoter and transcription termination signal (ϕ) flanking the CAT gene (black bars) interrupted by the 2080-bp *Eco*RI fragment of the *Kpn* I G-fragment of cowpox virus DNA (open bar). The *Alu* I–*Xba* I fragment containing the AX element is within this fragment.

DNA of plasmid p2098 after it had been linearized with *Nco* I. This generated 2.7-kilobase (kb) RNAs containing the interrupted CAT gene. Cleavage of these RNAs was assayed at 30°C in 25-μl reaction mixtures containing 40 mM Tris·HCl (pH 7.9); 40 mM KCl; 6 mM NaCl; 7.6 mM $MgCl_2$; 0.08 mM EDTA; 0.8 mM dithiothreitol; 1.2 mM ATP; 0.12 mM GTP, CTP, and UTP; 3 mM creatine phosphate; 1.2 mM spermidine; 6% (vol/vol) glycerol; 8–10 μg of protein (from 2.5 μl of whole-cell extract); and 1.5 μg of substrate RNA. RNAs were recovered by ethanol precipitation after proteins had been removed by phenol/chloroform extraction. Before RNA analyses, DNA was removed with RNase-free DNase I. The ^{32}P-labeled RNAs were resolved by electrophoresis in 1.6% agarose gels containing 2.2 M formaldehyde and were visualized by autoradiography of the dried gels. Reovirus single-stranded RNAs used as size standards were synthesized *in vitro* as described (23).

RNA Analyses. Nuclease S1 protection analyses were used to characterize the structure of the 5′ and 3′ ends of cleaved RNAs. Reaction conditions were as described (12), except that nuclease S1 was used at a concentration of 1000 units/ml. Primer extension analyses (12, 24) were used to characterize the structures of RNA 5′ ends.

RESULTS

RNA 3′ End Formation Is Not Dependent upon Either the Promoter or the RNA Polymerase. A cis-acting element directing the site-specific formation of the 3′ end of the mRNAs encoding the ATI protein has been identified within the

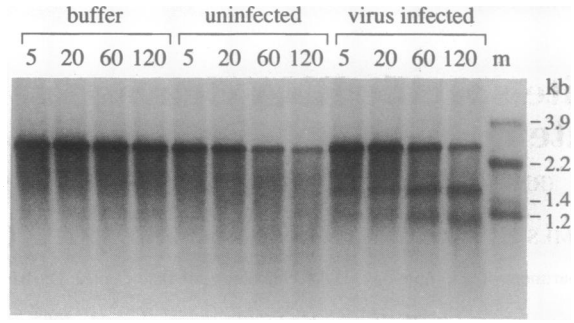

FIG. 2. RNA 3′ end formation *in vitro* is dependent upon an activity present in extracts of virus-infected cells. ^{32}P-labeled 2.7-kb RNAs transcribed from *Nco* I-linearized p2098 were incubated in reaction mixtures containing buffer alone or buffer supplemented either with extract of uninfected HeLa cells or with extract prepared from HeLa cells 15 hr after they had been infected with vaccinia virus. After incubation for 5, 20, 60, or 120 min, equal volumes were removed from each reaction mixture. RNAs were recovered from each sample and then resolved by agarose/formaldehyde gel electrophoresis. ^{32}P-labeled RNAs were visualized by autoradiography. The incubation period is indicated above each lane; lane m contained single-stranded RNA standards.

345-bp *Alu* I–*Xba* I fragment containing the sequence corresponding to the 3′ end of the mRNA (S. T. Howard, C.A.R., D.D.P., J.B.A., and D.J.P., unpublished data). When a DNA fragment containing this element (designated the AX element) was placed downstream of a CAT gene under the control of a T7 RNA polymerase promoter (within the thymidine kinase gene) in the genome of vaccinia virus A461, it directed the formation of late RNAs of a defined length that suggested these RNAs were transcribed from the promoter of the L4R gene (Fig. 1). This demonstrated both that the AX element functions in vaccinia virus as well as cowpox virus, and that it functions downstream of late promoters other than that of the gene encoding the ATI protein. Moreover, when cells were coinfected with vaccinia virus vTF7-3, which synthesizes the T7 RNA polymerase, additional CAT transcripts were formed (Fig. 1). Allowing for the additional length caused by the polyadenylylation of these RNAs, the 3′ ends of these RNAs corresponded either to the position of the T7 transcriptional terminator or to the position of the AX element. In contrast, *in vitro* transcription of the identical DNA template with T7 RNA polymerase failed to generate any CAT transcripts with defined 3′ ends other than those corresponding to the site of the T7 transcriptional terminator (data not shown). This indicated that the AX element did not act as a transcriptional termination signal for the T7 RNA polymerase. Significantly, these results demonstrated that the AX element could direct RNA 3′ end formation efficiently in cells whether the RNA was generated by the multisubunit RNA polymerase of the poxvirus or by the single-polypeptide RNA polymerase of phage T7.

The 3′ Ends Are Generated by Site-Specific RNA Cleavage. The lack of dependence upon either promoter or RNA polymerase suggested that the site-specific 3′ end formation might occur by a posttranscriptional mechanism. To test this hypothesis, *in vitro* synthesized RNAs containing the AX element were added to extracts of uninfected or virus-infected cells to assay for 3′ end formation dependent upon the AX element. The ^{32}P-labeled RNA substrate used in these assays was a 2.7-kb *in vitro* transcription product of the T7 promoter–CAT gene construct present in plasmid p2098. This *in vitro* synthesized RNA was equivalent to the 3.0-kb RNA generated by T7 RNA polymerase in the cells (Fig. 1). When the 2.7-kb RNA substrate was incubated in the presence of an extract of HeLa cells infected with vaccinia virus it was converted into two fragments, one about 1.1 kb long and the

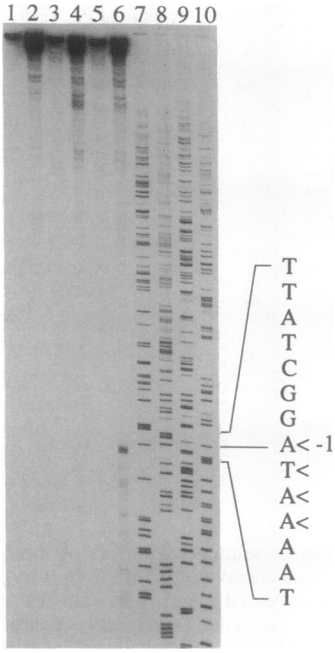

FIG. 3. Nuclease S1 protection analysis of the 3′ ends of RNAs generated by cleavage *in vitro*. Unlabeled 2.7-kb RNAs (p2098-derived) were incubated for 60 min either in extracts of uninfected HeLa cells or in extracts of HeLa cells prepared 15 hr after they had been infected with vaccinia virus. After extraction from the reaction mixtures these RNAs, and also untreated 2.7-kb RNAs, and yeast tRNAs, were separately annealed to a ^{32}P-labeled probe DNA consisting of a 1650-bp *Hin*PI–*Bam*HI fragment (3′ end-labeled at the *Hin*PI site) containing the sequence corresponding to the position of the RNA 3′ end site. Residual single-stranded nucleic acids were digested with nuclease S1. Nuclease-resistant products were resolved by electrophoresis in a 5% polyacrylamide gel containing 8.3 M urea. The ^{32}P-labeled DNA was visualized by autoradiography. Lanes 1–6 contained portions of the DNA probe protected by hybridization to yeast tRNAs (lane 1), untreated 2.7-kb RNAs (lane 2), endogenous RNAs present in the extract of uninfected cells (lane 3), the 2.7-kb RNAs after incubation in the extract of uninfected cells (lane 4), endogenous RNAs present in the extract of virus-infected cells (lane 5), and the 2.7-kb RNAs after incubation in the extract of virus-infected cells (lane 6). Lanes 7–10 contained the products of chain-termination sequence reactions using M13mp18 single-stranded DNA as the template, coelectrophoresed to provide size markers. The probe DNA sequence containing the nucleotide (designated −1) complementary to that at the 3′ end of the RNA is indicated. Arrowheads indicate the major endpoints of complementarity between the probe and the cleaved RNAs.

other about 1.6 kb long (Fig. 2). Similar conversion was not detected when the RNA was incubated either in buffer alone or in extracts from uninfected cells, suggesting that the conversion required a virus-induced factor. Moreover, the sizes of the fragments generated in the extracts of virus-infected cells suggested that these RNAs were generated by endoribonucleolytic cleavage of the 2.7-kb RNA at a position within the AX element in the RNA. An RNA cleavage at this site would generate the 3′ end of a 1.6-kb RNA and the 5′ end of a 1.1-kb RNA.

Nuclease S1 protection analyses confirmed the generation of RNA 3′ ends corresponding to a site within the AX element (Fig. 3). Endpoints of complementarity between the probe and the RNA, which corresponded to a region in the AX element about 130 nucleotides downstream of the *Alu* I recognition sequence, were readily detectable after the 2.7-kb RNAs had been incubated in the presence of an extract of virus-infected cells (Fig. 3, lane 6). However, similar endpoints of complementarity were not detected after the RNA substrate had been incubated in extracts of uninfected

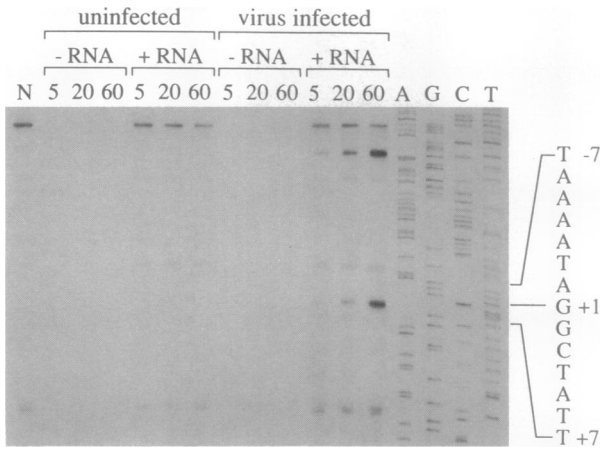

FIG. 4. Primer extension analysis of the 5′ ends of RNA products generated by cleavage *in vitro*. Unlabeled 2.7-kb RNAs (p2098-derived) were incubated for 5, 20, or 60 min either in extracts of uninfected HeLa cells or in extracts of HeLa cells prepared 15 hr after they had been infected with vaccinia virus. Oligonucleotides (5′-CGGGATCCGTAACGAAACATCCATCG-3′), labeled at their 5′ ends with ^{32}P, were annealed to RNAs recovered either from these reaction mixtures (+RNA) or from unsupplemented extracts (−RNA). After DNA synthesis, the cDNA products were resolved by electrophoresis in a 6% polyacrylamide gel containing 8.3 M urea and were visualized by autoradiography. Lane N, products of DNA synthesis using untreated 2.7-kb RNAs as substrate; lanes A, G, C, and T, products of sequence reactions that were coelectrophoresed to provide size markers. The predicted sequence of the cDNA containing the nucleotide (designated +1) at the first major endpoint of cDNA synthesis is indicated.

cells (Fig. 3, lanes 4). This result indicated that a factor in the virus-infected cells generated the formation of a novel RNA 3′ end whose sequence was 5′-AUUUUAU-3′. The position of this endpoint was consistent both with the predicted site of the 3′ end of the 1.6-kb RNAs and with the nucleotide sequence at the 3′ end of mRNAs encoding the ATI protein (S. T. Howard, C.A.R., D.D.P., J.B.A., and D.J.P., unpublished data).

The generation of 5′ ends corresponding to the sequence downstream of the predicted cleavage site within the AX element was detected by primer extension and nuclease S1 protection analyses. Extension of a primer, which annealed to the RNA substrate about 70 nucleotides downstream of the predicted RNA cleavage site, generated two cDNA products that were produced only if the RNA substrate had been incubated in the presence of extract of virus-infected cells (Fig. 4). The shorter of these two cDNA products was 98 nucleotides long, suggesting that it was derived from an RNA whose 5′ end mapped to the predicted site of RNA cleavage. In the RNA substrate, this sequence, 5′-CCGAUAA-3′, is immediately downstream of the sequence present at the 3′ ends of the 1.6-kb RNAs. The length of the other major cDNA product, about 168 nucleotides, indicated a cDNA 3′ end that did not correspond to either the position of any identified RNA 5′ ends or the position of any identified RNA 3′ ends. This suggested that the longer cDNAs may have been the products of second-strand cDNA synthesis. Consistent with this interpretation, nuclease S1 protection analysis (Fig. 5) identified only a single endpoint of complementarity between the 5′ ends of the RNA products and a probe extending 1.3 kb upstream of the position of the predicted RNA cleavage site. This single endpoint of complementarity corresponded closely to the position of the sequence 5′-CCGAUAA-3′, which the primer extension analysis (Fig. 4) had predicted to be at the 5′ end of the RNA.

Primer extension in the presence of dideoxynucleotides generated chain-terminated products corresponding to the

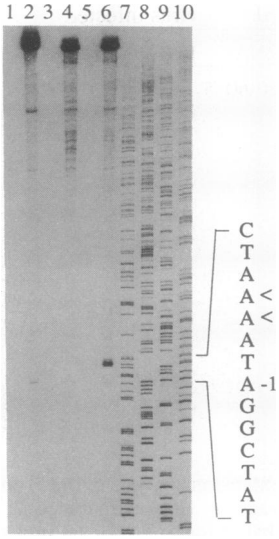

FIG. 5. Nuclease S1 protection analysis of the 5′ ends of RNAs generated by cleavage *in vitro*. Unlabeled 2.7-kb RNAs (p2098-derived) were incubated for 60 min either in extracts of uninfected HeLa cells or in extracts of HeLa cells prepared 15 hr after they had been infected with vaccinia virus. After extraction from the reaction mixtures these RNAs, and also untreated RNAs, untreated 2.7-kb RNAs, and yeast tRNAs, were separately annealed to ^{32}P-labeled probe DNA consisting of a 1455-bp *Eco*RI–*Xba* I fragment (5′ end-labeled at the *Xba* I site) containing the sequence corresponding to the position of the RNA 5′ end site. Residual single-stranded nucleic acids were digested with nuclease S1. Nuclease-resistant products were resolved by electrophoresis in a 6% polyacrylamide gel containing 8.3 M urea. The ^{32}P-labeled DNA was visualized by autoradiography. Lanes 1–6 contained portions of the DNA probe protected by hybridization to yeast tRNAs (lane 1), untreated 2.7-kb RNAs (lane 2), endogenous RNAs present in the extract of uninfected cells (lane 3), the 2.7-kb RNAs after incubation in the extract of uninfected cells (lane 4), endogenous RNAs present in the extract of virus-infected cells (lane 5), and, the 2.7-kb RNAs after incubation in the extract of virus-infected cells (lane 6). Lanes 7–10 contained the products of sequence reactions using M13mp18 single-stranded DNA as the template, which were coelectrophoresed to provide size markers. The sequence of the part of the DNA probe containing the nucleotide (designated −1) complementary to that at the 3′ end of the RNA is indicated. Arrowheads indicate the major endpoints of complementarity between the probe and the cleaved RNAs.

sequence of the partially cleaved RNA template (Fig. 6). This confirmed that the primer had annealed at a single position in the substrate RNA. The sequence analysis directly demonstrated that the sequence at the 5′ ends of the RNAs that were generated by incubation of the RNA substrate in extracts of virus-infected cells was 5′-CCGAUAA-3′. Furthermore, under these reaction conditions, and in the presence of 4 mM sodium pyrophosphate, which inhibits both second-strand cDNA synthesis and the premature termination of reverse transcription (24), significant quantities of the 168-nucleotide cDNAs were not generated.

The identification of novel 3′ and 5′ RNA ends corresponding to adjacent nucleotides in the substrate RNA within the AX element (Fig. 7) confirmed that a single endonucleolytic event generated the two RNA products. The identification of these cleavage products only after incubation of the substrate RNA in the presence of extracts of cells prepared during the late stages of viral replication suggests that the factor responsible for the RNA cleavage is either virus-induced or virus-encoded. Site-specific RNA cleavage was not observed in extracts prepared during the early stage of virus replication (unpublished results). When the RNA substrate was incubated in these extracts it was rapidly degraded.

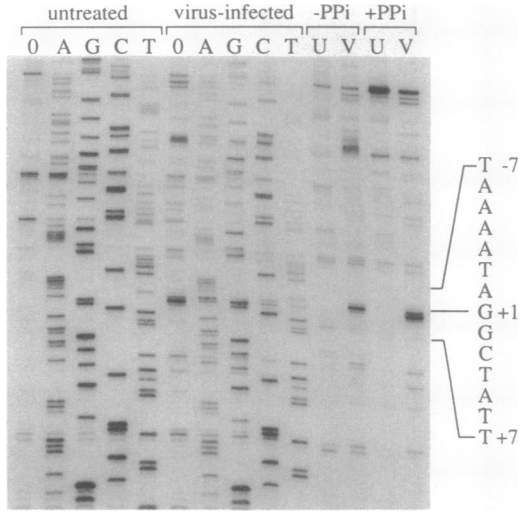

FIG. 6. Nucleotide sequence analysis of the 5′ ends of RNAs generated by *in vitro* cleavage. Conditions for primer extension analysis either of untreated 2.7-kb substrate RNAs or of substrate RNAs that had been incubated in extracts prepared from cells 15 hr after they had been infected with vaccinia virus were as described in Fig. 4, except that reactions were performed either in the absence of dideoxynucleotides (0), or in the presence of 10 μM dideoxy-ATP, -GTP, -CTP, or -TTP (A, G, C, and T). Primer extensions were also performed in the absence (−PPi) or presence (+PPi) of 4 mM sodium pyrophosphate, using the same RNA samples, designated U (untreated RNAs) or V (RNAs incubated in extracts of virus-infected cells). The concentration of each of the four dNTPs in these reactions was 500 μM. Products were resolved by electrophoresis in a 6% polyacrylamide gel containing 8.3 M urea and were visualized by autoradiography. The cDNA sequence containing the nucleotide (designated +1) at the first major endpoint of cDNA synthesis is indicated. The longer cDNAs are derived from the uncleaved RNA substrate.

DISCUSSION

The results demonstrate that the 3′ ends of the late mRNAs encoding the ATI protein are generated by site-specific cleavage of a primary RNA transcript. Cleavage occurs precisely after the sequence 5′-UUUUAU-3′ (Fig. 7), forming a new RNA 3′ end, which is then polyadenylylated. This mechanism of RNA 3′ end formation is distinct from that employed by the virus during the transcription of early genes, when the 3′ ends appear to be formed exclusively by the termination of transcription (5). The latter mechanism of RNA 3′ end formation is not operative at late times during viral replication (7–9). Therefore, it appears that the poxviruses employ at least two different mechanisms to generate the 3′ ends of their RNAs.

It is not known whether the 3′ ends of other viral RNAs are generated by site-specific RNA cleavage. With the exception of the telomeric RNAs (16), all other characterized viral RNAs appear to lack site-specific 3′ ends (10, 11). Although this may indicate that the 3′ ends of most viral late RNAs are formed by a different mechanism, the majority of viral late RNAs are <10 kb long (11, 25; unpublished data), which suggests that some form of transcriptional termination, or RNA cleavage, or a combination of the two, may generate the

	-15 ↓ +15
Primary RNA	5′-CACAAAAGAUUUUAUCCGAUAAUUCUUCAU-3′
mRNA 3′ end	5′-CACAAAAGAUUUUAUAAAAAAAAAAAAAAA$_{(n)}$

FIG. 7. The cleavage site in the AX element. The cleavage site in the primary RNA is indicated by the arrow. The 3′-polyadenylylated form of the mature RNA is shown beneath the sequence of the primary RNA.

3′ end of each late RNA. Therefore, the site-specific RNA cleavage mechanism generating the 3′ ends of the mRNAs encoding the ATI protein might represent a mechanism by which the 3′ ends of many of the late RNAs are formed. Differences in either the efficiency or the specificity of RNA cleavage among the various RNAs, or differences in the stabilities of the RNA products, might give rise both to a variety of 3′ ends in the transcripts of some genes and to more uniform 3′ ends in the transcripts of other genes. Whether the termination of transcription is linked to the cleavage of the nascent RNA, as may occur during the synthesis of mRNAs by RNA polymerase II (reviewed in ref. 1), remains to be determined.

The formation of site-specific 3′ ends in the viral mRNAs encoding the ATI protein resembles the process generating the 3′ ends of mRNAs in eukaryotic cells insofar as a cis-acting element in the primary transcript directs RNA cleavage, after which the nascent 3′ end is polyadenylylated (reviewed in ref. 1). In other respects, there are several differences between the two mechanisms of RNA 3′ end formation. (*i*) The formation of the 3′ ends of mammalian mRNAs involves interactions between several trans-acting factors and specific cis-acting elements that include both a polyadenylylation signal, whose consensus sequence is 5′-AAUAAA-3′, and a G+U-rich sequence that is downstream of the polyadenylylation site (reviewed in ref. 2). Similar cis-acting elements appear to be lacking in the regions flanking the cleavage site in the viral RNA. (*ii*) The formation of the 3′ ends of most mammalian mRNAs occurs in the nucleus of the cell, whereas the viral RNAs appear to be synthesized exclusively in the cytoplasm. (*iii*) The limited amount of mRNA 3′ end formation that does occur in the cytoplasm, such as that effecting the translational activation of maternal mRNAs during embryogenesis (26, 27), requires additional factors and the presence of a cytoplasmic polyadenylylation element, whose consensus sequence is 5′-UUUUUAU-3′ (28). A similar sequence is present at the cleavage site of the mRNA encoding the ATI protein (Fig. 7), but the proximity of this sequence to the cleavage site and the lack of other polyadenylylation signal sequences suggest that viral RNAs are neither cleaved nor polyadenylylated by this mechanism. (*iv*) The virus encodes a poly(A) polymerase that is capable of effecting the 3′ polyadenylylation of RNAs in the absence of specific polyadenylylation signals in the RNA substrate (29–32). These dissimilarities suggest that the virus and the cell employ different processes to generate the 3′ ends of their RNAs.

The nature of the factor effecting the site-specific cleavage of the viral RNAs has yet to be identified. Nonetheless, it is clear that this factor is either induced or activated during the course of viral replication. It is not detectable in extracts of uninfected cells, but it is present both in extracts of HeLa cells infected with vaccinia virus and in extracts of mouse L cells infected with vaccinia virus (unpublished results). It also appears to be present in human 143 cells infected with cowpox virus (12, 14) and in monkey kidney cells infected with vaccinia virus (15). The viral induction of this RNA cleavage activity in cells of different types and the propensity of the poxviruses to encode the enzymes that are required for the synthesis and processing of viral RNAs suggest that the factor involved may be encoded by the virus. However, the possibility that the factor is a host cell enzyme which is induced or activated by virus infection has not been ruled out.

Although it is unclear what factor is responsible for the site-specific cleavage of the viral RNAs, the results of this study have shown that this factor, together with the AX element, can be used to generate sequence-specific cleavage in RNAs other than those encoding the ATI protein. This provides a method of directing 3′ end formation in RNAs generated in cells infected with poxviruses, without the use of the phage T7 transcriptional apparatus or the inclusion of a self-cleaving ribozyme in the targeted RNA (20, 33).

Finally, because of the likelihood that viral factors are involved in the cleavage of viral RNAs, and because of the ease with which poxvirus genetic information can be manipulated, the poxviruses should provide a uniquely practical system for studies on endoribonucleolytic mechanisms of RNA 3′ end formation.

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