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Loss of Marek's Disease Virus Tumorigenicity Is Associated with Truncation of RNAs Transcribed within *Bam*HI-H

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The attenuation of Marek's disease virus (MDV) is associated with loss of pathogenicity and tumorigenicity. Previous studies have demonstrated a strong correlation between attenuation and amplification of a specific sequence located within the MDV terminal and internal repeats. We recently reported that the regions containing the amplified sequences, the *Bam*HI D and H fragments, were transcriptionally active. However, differential transcription activity was observed to exist between attenuated and pathogenic MDV strains. Specifically, a major transcript of 1.8 kilobases was found to be produced by pathogenic MDV and not by attenuated MDV. We now report that the disappearance of this transcript is concomitant with the production of a 0.4-kilobase RNA, an RNA resulting from the truncation of the tumorigenicity-related transcript.

Marek's disease virus (MDV) is an avian herpesvirus which induces lymphoproliferative disease and demyelination of peripheral nerves in the chicken. The virus-induced disease, Marek's disease, was one of the first neoplastic diseases found to be caused by a herpesvirus. Marek's disease later became the first naturally occurring lymphomatous disorder to be effectively controlled by vaccination (13, 31, 33).

Several investigators (10, 18, 29) demonstrated that serial in vitro passage of virulent MDV in primary chicken embryo fibroblasts (CEF) resulted in a loss of virion tumorigenicity. This loss of tumorigenicity strongly correlated with an expansion in two particular regions of the viral genome (18, 26, 34). Experiments comparing the structures of viral DNA from pathogenic and nonpathogenic strains of MDV revealed that a 1.5-kilobase-pair (kbp) *Bgl*II-*Pst*I subfragment of *Bam*HI-D and -H, present in the TR_L and IR_L, respectively (Fig. 1), exhibited size heterogeneity (expansion) only in the nonpathogenic strains of virus (18). It was later discovered that this heterogeneity was due to the amplification of a 132-bp repeat sequence found within BamHI-D and -H (26). Since amplification of this region was only detected within nonpathogenic strains of MDV, the data suggest that *Bam*HI-D and -H contain one or more genes responsible for viral tumorigenicity.

We recently reported that this region of MDV is transcriptionally active and presented a map delineating the transcripts produced (8). Within this region, a 1.8-kilobase (kb) rightwardly transcribed RNA is produced in CEF infected by pathogenic strains of MDV and in cell lines established from MDV-induced tumors. This RNA is not detected in CEF infected by attenuated strains of MDV. These results suggest that this gene is associated with the tumorigenicity of MDV. We now report that the disappearance of this RNA, associated with attenuation and the loss of tumorigenicity, is due to truncation of this 1.8-kb transcript.

MATERIALS AND METHODS

Virus strains. The pathogenic strain of MDV used in this study, RBIB, was provided by L. W. Schierman, University of Georgia, Athens. The nonpathogenic strains used were CVI-988 (33), JM102/40D (provided by R. L. Witter, U.S. Department of Agriculture, East Lansing, Mich.), and GA clone 19-17, a subclone of the pathogenic strain GA (14).

Preparation of cellular DNA and RNA from CEF infected with MDV. Primary CEF cell cultures, prepared from specific-pathogen-free White Leghorn embryos (SPAFAS Inc., Norwich, Conn.) were infected by cocultivation with MDV-infected CEF at a ratio of 10 parts CEF to 1 part virus-infected CEF. The cells received Eagle minimal essential medium containing 1% newborn calf serum and were harvested when 80% of the cells showed cytopathic effect.

Total cellular DNA was recovered by treating the cells with pronase-sodium dodecyl sulfate followed by phenol extraction as described previously (18). Total RNA was prepared by the method of Chomczynski and Sacchi (9). Polyadenylated [poly(A)$^+$] RNA was isolated from total cytoplasmic RNA by oligo(dT)-cellulose chromatography (3).

Southern and Northern (RNA) blot hybridization. Total infected-cell DNA was digested with restriction enzymes following the recommendations of the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). DNA fragments were electrophoresed in agarose gels and then transferred to nylon membranes (Schleicher & Schuell, Inc., Keene, N.H.) by the method of Southern (36). Hybridizations were done under conditions described previously (18) in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–6× Denhardt solution (1× Denhardt solution is 0.05% bovine serum albumin, 0.05% Ficoll, 0.05% polyvinylpyrrolidone [12])–50% formamide at 45°C for ^{32}P-oligolabeled DNA probes (16). Autoradiography was performed at −80°C with Kodak X-OMAT AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) and an intensifying screen.

Poly(A)$^+$ RNA was denatured with 1 M glyoxal in aqueous 50% dimethyl sulfoxide by the method of McMaster and Carmichael (28) and electrophoresed in agarose. Chicken 28S and 18S RNAs were used as size markers.

PCR for probe preparation. The oligonucleotides GGG TGAGCCAATCGGATATG and GTAGTTTATTCATTA were synthesized with the Applied Biosystem 380B DNA

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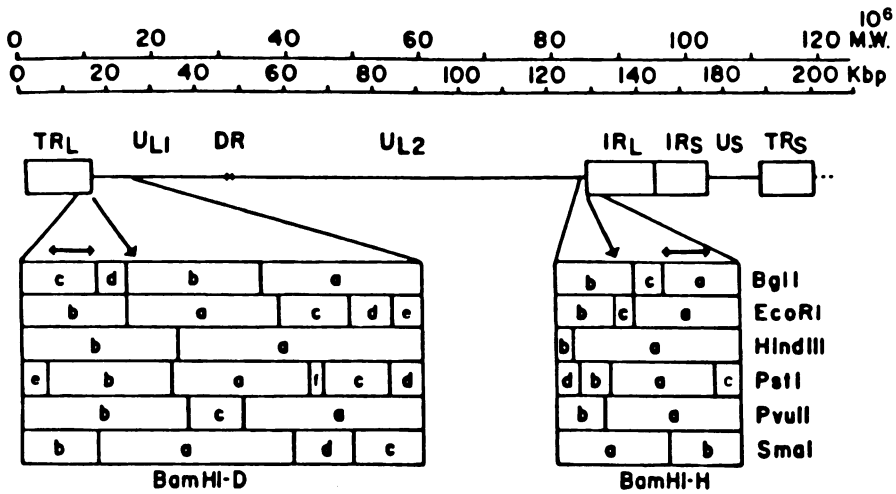


FIG. 1. Schematic representation of the MDV genome. The 120-kilodalton genome of MDV is shown along with the location and restriction maps of the *Bam*HI D and H fragments. The single-headed arrows indicate the location of the junction regions of the terminal (TR_L) and internal (IR_L) repeats with the long unique regions U_{L1} and U_{L2}, respectively. The double-headed arrows locate the region containing the sequences which undergo amplification upon repeated passage in vitro. The direct repeat (DR), short internal repeat (IR_S), short terminal repeat (TR_S), and short unique (U_S) repeat regions are shown.

synthesizer (Forest City, Calif.). The polymerase chain reaction (PCR) was performed by the protocol provided with the GeneAmp Kit produced by Perkin-Elmer Cetus (Norwalk, Conn.). The resulting amplified product was isolated by electroelution into a trough cut into the agarose gel (25) after electrophoresis and radiolabeled as described below.

S1 analysis of viral mRNA. Viral poly(A)$^+$ RNA from infected CEF was analyzed by the method of Berk and Sharp (6). The DNA fragment, generated by PCR, used to detect the 5′ end of the mRNA was labeled at the 5′ termini following removal of the terminal phosphate with bacterial alkaline phosphatase. The terminal phosphate was subsequently replaced with T4 polynucleotide kinase and [γ-^{32}P]ATP (3,000 Ci/mmol; Du Pont NEN Research Products, Boston, Mass.). The *Dde*I-*Dde*I DNA fragment, whose 5′ end is located approximately 2 to 5 nucleotides downstream from the transcription initiation site of the 1.8-kb RNA, was used to detect the 3′ end of the mRNA. This fragment was labeled at the 3′ termini with [α-^{32}P]dTTP (3,000 Ci/mmol; NEN Research Products) and the Klenow fragment of *Escherichia coli* DNA polymerase I (25). Approximately 50 ng (2×10^4 to 1×10^5 cpm) of the DNA probe was precipitated with 20 μg of poly(A)$^+$ RNA combined with enough *E. coli* tRNA to achieve a total of 200 μg of nucleic acid. As a negative control, the probe was precipitated along with 200 μg of *E. coli* tRNA. The resulting pellets were dried and suspended in 30 μl of hybridization buffer (40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.4], 1 mM EDTA, 0.4 M NaCl, 80% formamide). The mixture was placed in a water bath at 80°C for 10 min and then immediately transferred to a water bath at 42 to 48°C and allowed to hybridize for 3 h. After hybridization, 0.3 ml of ice-cold S1 nuclease buffer (280 mM NaCl, 50 mM sodium acetate [pH 4.6], 4.5 mM $ZnSO_4$, 1,000 U of S1 nuclease per ml, and 20 μg of heat-denatured salmon sperm DNA per ml) was added, mixed, transferred to a 45°C water bath, and incubated for 1 h. The reaction was terminated by the addition of 66 μl of 3.0 M ammonium acetate (pH 5.2). The sample was extracted once with phenol-chloroform and once with chloroform, and the protected fragments were precipitated with an equal volume of isopropanol. The precipitate was pelleted, dried, suspended in 5 to 10 μl of loading buffer (90% formamide, 1× TBE [pH 8.3; 50 mM Tris hydrochloride, 50 mM boric acid, 1 mM EDTA], 0.02% bromophenol blue, 0.02% xylene cyanol) and heated at 80°C for 5 min. The samples were subjected to electrophoresis through a 5% polyacrylamide–7 M urea gel to size the S1-protected fragments.

RESULTS

Differential transcription from pathogenic and attenuated virus strains. Northern blots of total-cell RNA from CEF infected with the tumorigenic MDV strain RBIB (passage 14) or infected with the attenuated vaccine strain, CVI-988, were hybridized with a series of radiolabeled DNA fragments. These DNA fragments together spanned the entire *Bam*HI-H region (Fig. 2). There appeared to be no qualitative difference in the hybridization patterns generated by the RNA from the pathogenic and attenuated virus when hybridized with probes I and II (Fig. 2, lanes I and II). However, when these RNAs were hybridized with probes III and V, significant qualitative differences reflecting detection of different RNA fragments were observed (Fig. 2, lanes III and V).

As has been reported previously (8), probe III detected the leftwardly transcribed 3.8- and 1.8-kb RNAs produced by both the attenuated and pathogenic virus as well as the rightwardly transcribed 1.8-kb RNA produced only by the pathogenic virus. With this probe, a major transcript of 0.4 kb was observed to be produced by CVI-988, while a lesser amount of this 0.4-kb RNA was detected in the RNA produced by the pathogenic strain of MDV (Fig. 2, lane III). At this time, we believe that the low level of 0.4-kb RNA generated by RBIB represents transcription from a small subpopulation of virus having already undergone genomic amplification and attenuation. This hypothesis is supported by the observation that when RNA isolated from MDV-infected lymphoblastoid cell lines induced with iododeoxyuridine (cell lines containing virus that does not exhibit amplification of the 132-bp repeats) was hybridized with probe III, the 0.4-kb RNA was not detected (data not shown).

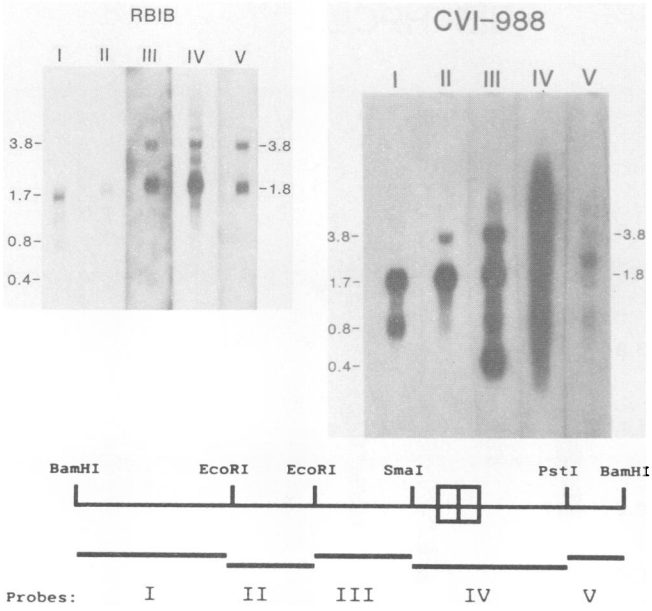


FIG. 2. Northern blot hybridization studies of pathogenic and attenuated virus strains. Northern blots of poly(A)$^+$ RNA isolated from the pathogenic strain RBIB (passage 14) or the attenuated strain CVI-988 of MDV were hybridized with subfragment probes which together spanned the entire *Bam*HI-H fragment (bottom). Lanes I to V represent hybridization with probes I to V, respectively. Restriction enzyme sites are labeled as follows: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sma*I. The boxes represent two copies of the 132-bp direct repeat sequence. The sizes of the RNAs are shown (in kilobases).

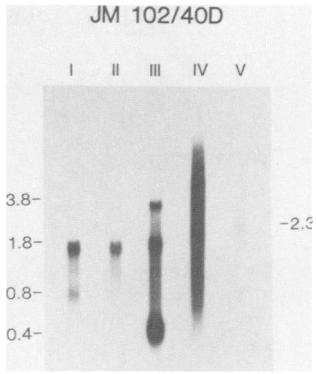


FIG. 3. Northern blot hybridization of the attenuated strain JM102/40D. Northern blots of poly(A)$^+$ RNA from JM102/40D were hybridized with probes which together spanned the entire *Bam*HI H fragment (described in the legend to Fig. 2). Lanes I to V represent hybridization with probes I to V, respectively. The sizes of the RNAs are shown (in kilobases).

Probe IV detected a heterogeneous population of RNAs produced in cells infected with the pathogenic virus as well as with the attenuated virus (Fig. 2, lane IV). Probe V detected the rightwardly transcribed 3.8- and 1.8-kb RNAs produced by the pathogenic virus but detected only a 2.5-kb RNA produced by the attenuated virus.

To demonstrate that the differences in the transcription patterns observed were associated with the attenuation process, Northern blot hybridization studies were repeated with RNA from CEF infected with the attenuated viral strain JM102/40D (Fig. 3). Analysis of the resulting Northern blot hybridization confirmed that the emergence of the 0.4-kb RNA (lane III) and the disappearance of the 3.8- and 1.8-kb RNAs (lane V) were indeed associated with the attenuation process. The attenuated strain, GA clone 19-17, also produced the 0.4-kb RNA (data not shown).

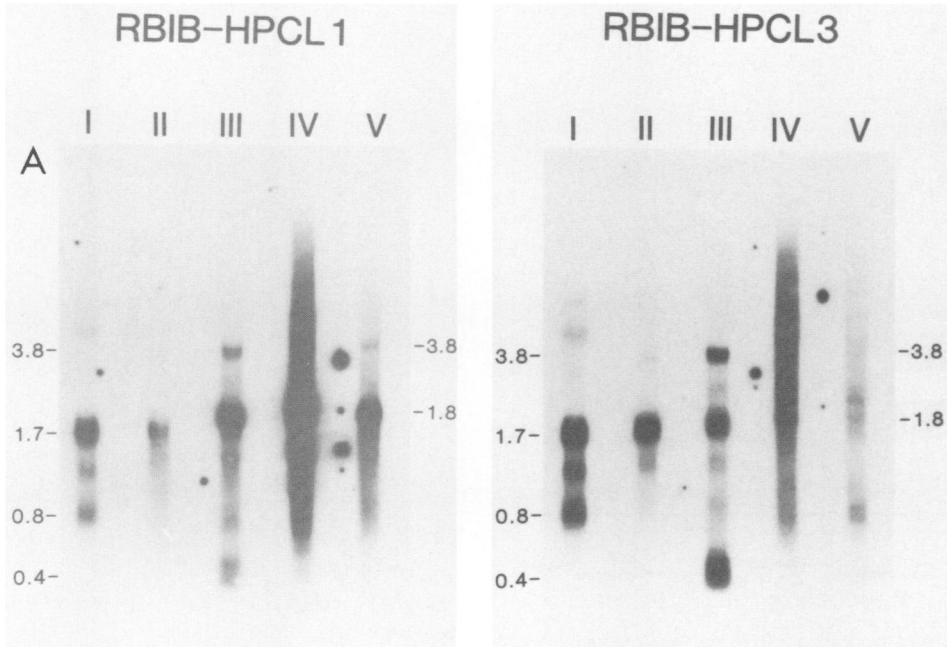
To verify further the association of differential transcription with the attenuation process, we attenuated the pathogenic strain, RBIB, by repeated passage through CEF. At passage 64, several individual foci were isolated and passaged as subclones. The viral DNA was isolated from six of these subclones, and the extent of amplification of the 132-bp repeats was analyzed via Southern blot hybridization as described previously (8). Two of the virus subclones (subclones 1 and 3) were selected for further investigations. The viral DNA from high-passage subclone 1 (HPCL 1) contained an average of 8 copies of the 132-bp repeat, while the DNA from high-passage subclone 3 (HPCL 3) contained an average of 30 copies of the 132-bp repeat (data not shown). Northern blot hybridization studies were repeated with poly(A)$^+$ RNA from CEF infected with the RBIB high-passage subclones (Fig. 4A).

Fortuitously, RBIB HPCL 1 exhibited a transcriptional pattern intermediate between those of the pathogenic parental strain, RBIB, and the attenuated strains, CVI-988 and JM102/40D. The intensity of the 1.8-kb band in lane III and the presence of a 1.8-kb band in lane V suggested that the rightwardly transcribed 1.8-kb RNA was produced by the majority of virus within what appears to be a heterogeneous virus population. However, the presence of the 0.4-kb RNA in lane III suggested that a small population of virus within this subclone was producing a transcriptional pattern similar to that of the attenuated strains.

The other high-passage virus subclone, RBIB HPCL 3, produced a hybridization pattern mimicking the pattern produced by the attenuated strains (Fig. 4A). The intensity of the 0.4-kb band in lane III and the disappearance of the 3.8-kb and the 1.8-kb RNAs in lane V suggested that the vast majority if not the total MDV population in RBIB HPCL 3 consisted of virus that produced transcripts indicative of the attenuated strains rather than the parental pathogenic strain, RBIB.

The altered transcription pattern in the RBIB high-passage clones is clearly demonstrated in Fig. 4. A PCR-generated DNA fragment containing sequences homologous to the 5′ end of the rightwardly transcribed 1.8-kb RNA was used to probe the RNA isolated from CEF infected with HPCL 1 and 3. The resulting Northern blot hybridization of RNA from HPCL 1 showed that an approximately equal amount of the 1.8-kb and the 0.4-kb RNAs was transcribed from the population of virus within this subclone. Contrastingly, the population of virus in HPCL 3 was observed to produce only the 0.4-kb RNA.

Transcription initiation and termination of the 0.4-kb RNA. S1 nuclease protection assays were performed to determine whether the 0.4-kb RNA produced by the attenuated strains of MDV resulted from truncation of the 1.8-kb RNA. As the transcription initiation site of the 1.8-kb RNA produced by pathogenic virus was determined previously (8), a DNA fragment spanning the −130 and +117 region of the gene was generated by PCR. The resulting fragment was 5′-end labeled, hybridized to RNA from pathogenic and attenuated strains of MDV, and then digested with S1 nuclease. RNA from both the pathogenic and attenuated strains of MDV



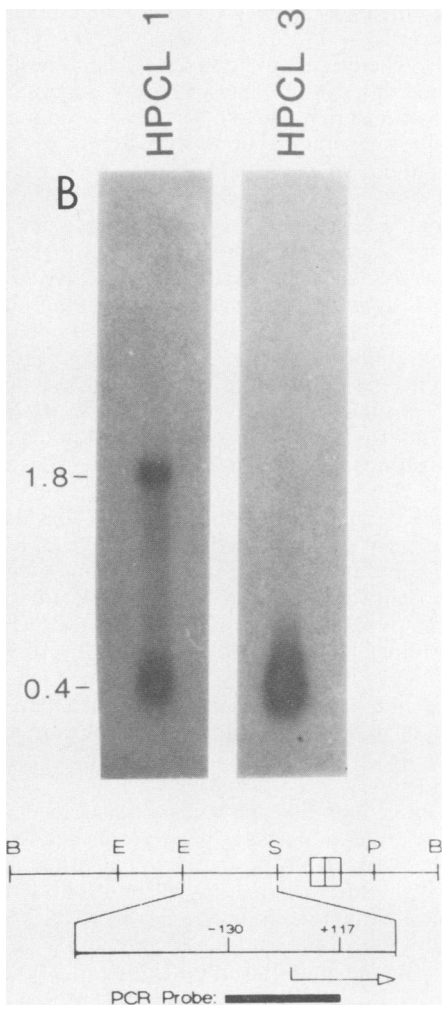


FIG. 4. Northern blot hybridizations of the RBIB high-passage clones HPCL 1 and HPCL 3. (A) Northern blots containing poly(A)$^+$ RNA generated by HPCL 1 and HPCL 3 were hybridized with the probes described in the legend to Fig. 2. Lanes I to V were hybridized with probes I to V, respectively. The sizes of the RNAs are shown (in kilobases). (B) Northern blots of poly(A)$^+$ RNA from RBIB HPCL 1 and HPCL 3 were hybridized with a PCR-generated fragment. The location of the 247-bp PCR-generated probe within the *Bam*HI H fragment is shown at the bottom. Restriction enzyme sites are labeled as follows: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sma*I. The boxes represent two copies of the 132-bp direct repeat sequence. The sizes of the RNAs are shown (in kilobases).

protected a fragment of 118 nucleotides (Fig. 5). These results indicate that both the pathogenic and attenuated virus strains produce transcripts initiating from the same loci.

When the 3′-end-labeled 1.35-kb *Dde*I fragment was used in an S1 nuclease protection assay, the 3′ end of the 0.4-kb transcript was located approximately 200 nucleotides from the transcription initiation site (Fig. 6). Considering the inability of fragments immediately downstream of this region to hybridize to the 0.4-kb RNA, the lack of an appropriate splice signal sequence, along with previous observations that MDV transcripts contain poly(A) tails of approximately 200 nucleotides, we believe that the 0.4-kb RNA is a truncated version of the 1.8-kb RNA transcribed by tumorigenic virus.

Analysis of the termination region. Computer analysis of the sequence surrounding the truncation site was performed with the integrated software package PCGENE (IntelliGenetics, Mountain View, Calif.). Several potential polyadenylation signals (11) were found at locations 50 nucleotides (AATAGA), 73 nucleotides (AATAAA), 99 nucleotides (AATAAA), and 106 nucleotides (AATAAG) 5′ of the termination site (Fig. 7A). The truncation site was found to be G+T rich (10 of the 13 nucleotides immediately surrounding the site were G or T). The region immediately 3′ (<10 nucleotides) of the termination site was found to contain overlapping sequences capable of forming energetically stable loop-stem structures (−14 and −13.6 kcal) (Fig. 7B).

The termination of this truncated transcript was found to preclude the possible expression of an open reading frame

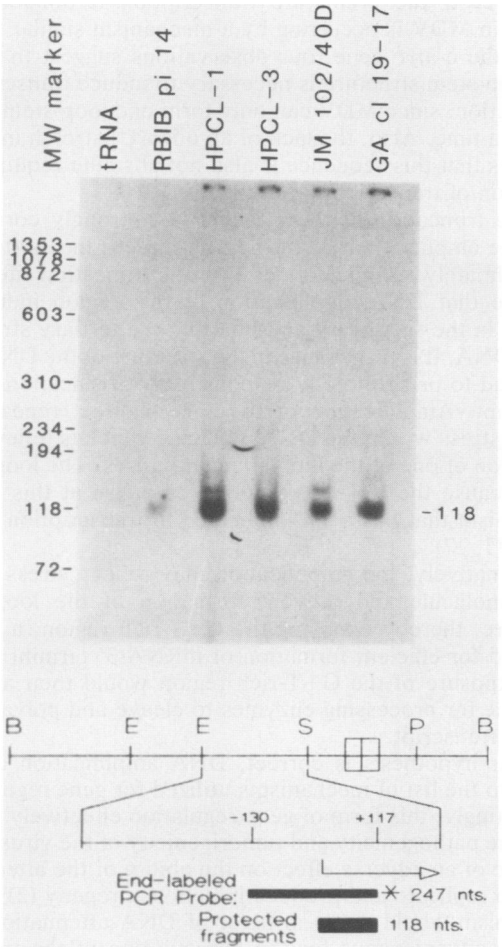


FIG. 5. Pathogenic and attenuated virus strains utilize the same transcription initiation site. The PCR-generated 247-bp fragment, shown at the bottom, was hybridized with RNA from the pathogenic strain RBIB (lane 2), several attenuated strains of MDV (lanes 3 to 6), or *E. coli* tRNA (lane 1). The hybrids were then treated with S1 nuclease, and the products were electrophoresed through a denaturing 5% acrylamide gel. A single fragment of 118 nucleotides was protected by the RNA from all strains of MDV. The location of the protected fragment within *Bam*HI-H is shown at the bottom. Several restriction sites are shown: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; and S, *Sma*I. The rectangles indicate the position of the 132-bp repeats. *Hae*III-digested ϕX174 was used as a molecular weight marker, and the sizes are indicated (in nucleotides).

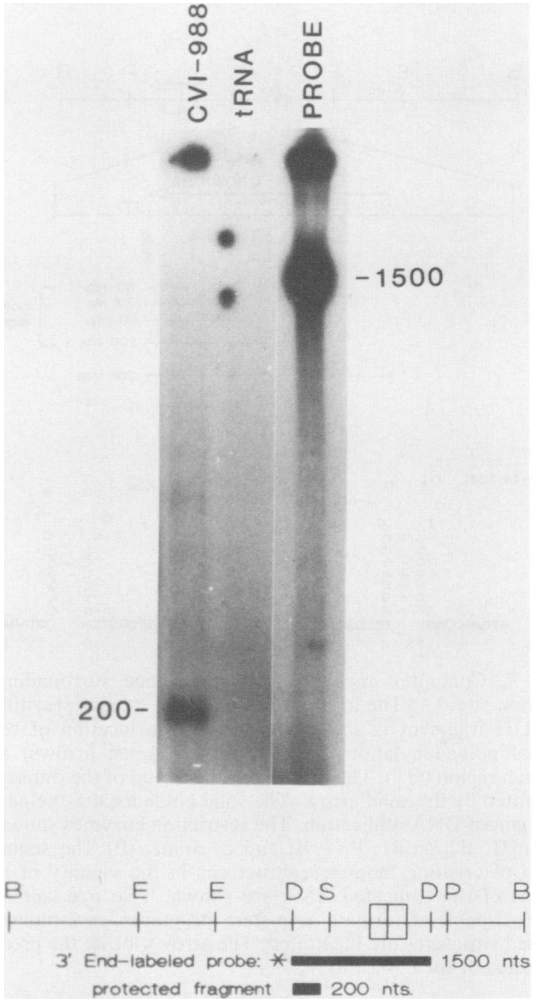


FIG. 6. Locating the 3′ end of the 0.4-kb RNA produced by attenuated MDV. The 3′-end-labeled 1,500-bp *Dde*I-*Dde*I fragment was hybridized with RNA from CVI-988 or with *E. coli* tRNA. The hybrids were digested with S1 nuclease and electrophoresed through a denaturing 5% acrylamide gel. A single protected fragment of 200 nucleotides (nts.) was detected. The locations of the probe and the protected fragment within *Bam*HI-H are shown at the bottom. Restriction enzyme sites shown are: B, *Bam*HI; D, *Dde*I; E, *Eco*RI; P, *Pst*I; and S, *Sma*I.

which could code for a protein of 63 amino acids. This open reading frame is contained within the 1.8-kb RNA that is produced by tumorigenic MDV. The 0.4-kb RNA would encode only three or four amino acids of this polypeptide. A search for homology to the predicted protein against the sequences of known proteins revealed strongest homologies to a retrovirus-related *gag* polyprotein (37.5% identity in a 24-residue overlap) and a kinase-related transforming protein (41.2% identity in a 17-residue overlap). Computer analysis by the method of Klein et al. (22) predicted that this 63-amino-acid polypeptide would be an integral protein with a single segment (amino acids 31 to 47) that would span the membrane.

DISCUSSION

The strong correlation between attenuation and the amplification of sequences within the *Bam*HI H fragment led to the development of our hypothesis that this amplification process affects the regulation of a gene or genes within this region. We recently reported that this region was transcriptionally active in pathogenic and attenuated virus strains. However, pathogenic and attenuated MDV strains displayed differential transcription activity in this region of the genome. More specifically, a rightwardly transcribed 1.8-kb RNA produced by pathogenic strains of MDV was absent in cells infected by the attenuated strains of MDV. The data presented in our current study show that the loss of the 1.8-kb RNA is concomitant with the appearance of a 0.4-kb RNA, a transcript that arises from truncation of the 1.8-kb RNA.

Premature or alternative transcription termination which generates truncated transcripts has been known to be a regulatory mechanism for genes in procaryotes (2, 32, 38). Premature transcription termination has recently been demonstrated to be a mechanism for controlling the expression

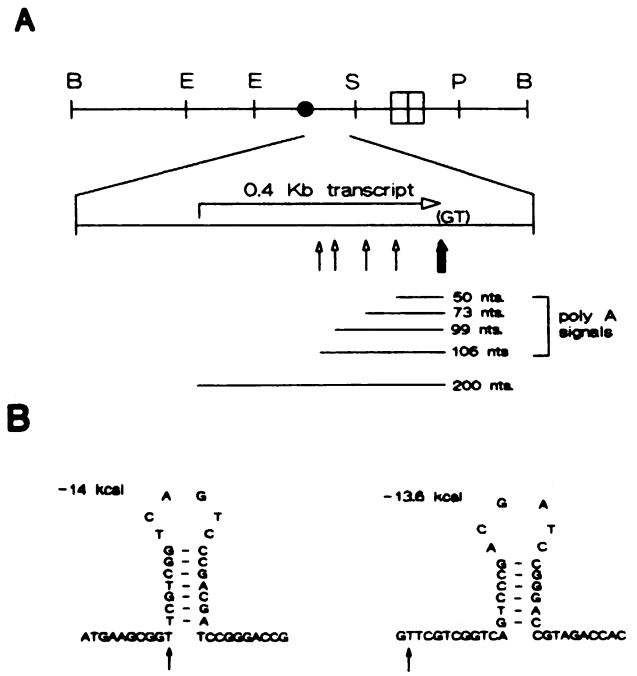


FIG. 7. Computer analysis of the sequence surrounding the truncation site. (A) The location of the 0.4-kb transcript within the *Bam*HI H fragment is shown along with the location of several potential polyadenylation (poly A) signals (open arrows) and a G+T-rich region (GT). The location of the 3′ end of the transcript is represented by the solid arrow. The solid circle locates the putative lytic origin of DNA replication. The restriction enzymes shown are: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; and S, *Sma*I. (B) The sequences capable of creating loop-stem structures in the vicinity of the 3′ terminus of the truncated RNA are shown. Two overlapping sequences capable of forming loop-stem structures were found. The predicted structures are illustrated. The arrows locate the predicted 3′ terminus of the 0.4-kb RNA.

of mouse (30, 37) and human (4, 5, 15) c-*myc* genes as well as the simian virus 40 (SV40) VP1 gene (19, 20). The mechanisms that result in the premature termination of transcripts within the c-*myc* and SV40 VP1 genes have been elucidated and appear to be similar to each other and to the mechanisms utilized to control many procaryotic genes as well. The leader sequences of the c-*myc* and SV40 genes, like many found in procaryotic genes, are able to exist in two alternative configurations. These configurations consist of either a single loop-stem structure, which allows readthrough, or a pair of loop-stem structures, which participate in transcription termination (15). The termination of transcription in these genes is found to occur on the 3′ side of the second loop-stem structure at a stretch of oligo(U).

The region surrounding the 3′ end of the MDV 0.4-kb RNA contains overlapping sequences that are also capable of forming loop-stem structures. Since the sequences capable of forming the loop-stem structures overlap, only one of the loop-stems can exist at any specific time. Neither of these potential loop-stem structures contain the oligo(U) stretch common to the SV40 VP1 transcript, the c-*myc* transcript, the bacteriophage lambda 6S transcript (35), and the *E. coli trp* transcript (24). However, the work performed by Bentley and Groudine (5) demonstrated that the oligo(U) stretch could be deleted without affecting the termination of the c-*myc* message. What did appear to be necessary was the retention of sequences capable of forming the 3′ loop-stem structure. If the premature transcription termination observed in MDV is occurring by a mechanism similar to that within the c-*myc* gene, our observations suggest that only one loop-stem structure is necessary to induce transcription termination, since MDV can only form one loop-stem structure at a time. Also, the lack of an oligo(U) stretch in MDV suggests that this sequence is also not absolute required for induction of transcription termination.

Since truncation of the 1.8-kb RNA strongly correlates with the amplification of the 132-bp direct repeats (located approximately 390 nucleotides 3′ of the truncation site), it is possible that DNA amplification of this region induces a change in the secondary and possibly the tertiary structure of the DNA. It is this change in the structure of the DNA that may lead to or be directly responsible for truncation of the transcript. Amplification of the 132-bp direct repeat may induce stress within the DNA molecule which is relieved by formation of one of the loop-stem structures. The loop-stem would cause the RNA polymerase to pause at this site, a mechanism that has been implicated in transcription termination (1, 17).

Alternatively, the amplification may relieve stress in the DNA molecule and prevent formation of the loop-stem structure, thereby exposing the G+T-rich region, a region required for efficient formation of mRNA 3′ termini (7, 23, 27). Exposure of the G+T-rich region would then make it available for processing enzymes to cleave and polyadenylate the transcript.

If our hypothesis is correct, DNA amplification can be added to the list of mechanisms utilized for gene regulation. Interestingly, this form of gene regulation effectively diminishes the pathogenicity and tumorigenicity of the virus in the absence of an adverse effect on the ability of the attenuated virus to replicate and produce infectious progeny (21). This mechanism would argue in favor of DNA attenuation as a process advantageous for the perpetuation of the virus as well as advantageous for the chicken.

This report, along with those published previously, supports the original hypothesis that this region of MDV is strongly associated with the tumorigenic potential of MDV. More recent investigations within our laboratory now indicate that two other transcripts initiating from the same start site as the 1.8-kb RNA are also truncated to the 0.4-kb RNA (G. Bradley, A. Tanaka, and M. Nonoyama, manuscript in preparation). Sequencing of the cDNAs corresponding to these transcripts will allow us to better predict the relationships between the loss of these RNAs and the loss of the tumorigenic potential of the virus. Also, the finding that truncation of the 1.8-kb RNA precludes the potential expression of a protein having homology to a transforming protein kinase necessitates further investigations into its biological functions.

The data reported here suggest that truncation of transcripts originating from within this region of the viral genome is a phenomenon associated with the attenuation process and strongly suggest that one or several of these RNAs is directly related to the tumorigenic potential of MDV.

ACKNOWLEDGMENTS

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