Latent Transcripts of Marek's Disease Virus Are Clustered in the Short and Long Repeat Regions

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Marek's disease herpesvirus (MDV) induces tumors in chickens, and lymphoblastoid cells derived from such tumors contain the viral genome in a latent state and do not produce infectious virus. Poly(A)⁺ RNAs extracted from MDV-induced kidney lymphoma cells and from MKT-1 cells, a nonproducing lymphoblastoid cell line derived from a tumor induced by MDV, were electrophoretically separated under denaturing conditions, transferred to a solid substrate, and hybridized with labeled DNA probes representing approximately 95% of the virus genome. These analyses revealed 29 viral RNA transcripts in the kidney lymphoma and 32 viral RNAs in MKT-1 cells. In both instances, the transcripts hybridized to a restricted region comprising approximately 20% of the MDV genome located in the repeats flanking the long and short unique sequences and in the adjacent unique sequences. The sizes of the transcripts derived from kidney lymphoma and MKT-1 cells and the distributions of the homologous regions in the viral genome were very similar. The most abundant transcripts were homologous to the BamHI I₂ fragment. The results suggest that expression of the MDV genome in MKT-1 cells closely reflects the expression of the MDV genome in tumors. The gene expression over extended regions of the genome is in concordance with that observed in latent gammaherpesviruses rather than that observed in latent alphaherpesviruses.

Marek's disease virus (MDV), an avian herpesvirus, causes lymphoproliferative disease in chickens, its natural host (5). Malignancies are apparent 4 to 6 weeks after infection. Rapid and reproducible induction of lymphomas, which follow inoculation with MDV, strongly suggests that MDV contains one or more genes that are responsible for tumor induction.

The MDV genome is present in tumors induced by MDV infection in chickens. These tumors are free of virus particles, thus indicating that the viral DNA found in these tumors must be present in a latent form (19). A lymphoblastoid cell line, designated MKT-1, has been established from Marek's disease kidney lymphoma in our laboratory. MKT-1 cells contain an average of 15 complete copies of the MDV genome per cell, the majority of which are maintained in an episomal state, but the cells do not produce infectious virus (25).

To date there has been no information on the number, identity, or function of viral genomes expressed in MDV-induced tumors, although studies have been reported for latently infected cells (17, 20). In this paper we report that MDV-induced kidney lymphoma in chickens and the MKT-1 lymphoblastoid cell line contains numerous transcripts that are homologous to specific and restricted domains of the MDV genome.

Relevant to this report are details of the structure of the MDV genome. MDV DNA extracted from virions is a linear, 180-kbp double-stranded molecule (14) and consists of two sets of unique sequences, a long and a short region, each flanked by inverted repeats designated as the terminal (TRL and TRS) and internal (IRL and IRS) repeats (6) (Fig. 1).

MATERIALS AND METHODS

Cells and virus strains. The T-lymphoblastoid cell line MKT-1 (MDCC-LSI) was derived from a kidney tumor and

was maintained in RPMI 1640 medium supplemented with antibiotics and 10% newborn calf serum and incubated at 37°C in an atmosphere of 5% CO₂. For lytically MDV-infected cells, a nonpathogenic strain, CVI-988, was propagated in primary chicken embryo fibroblasts (CEF) as described previously (10). Cells were harvested when 80% of cells showed cytopathic effects. Kidney lymphoma was collected from 5-week-old isolator-held SPAFAS-SPF chickens that had been inoculated intra-abdominally with 100 focus-forming units of RBIB, a pathogenic strain of MDV. Neither tumor tissues nor MKT-1 cells showed any evidence of viral antigen synthesis by indirect immunofluorescence staining.

Preparation of viral DNA and labeling of DNA probes. Cloned BamHI MDV plasmid DNA was isolated by the Hirt extraction method (11), precipitated with polyethylene glycol, and banded in CsCl gradients containing ethidium bromide as previously described (9). All of these BamHI-cloned library fragments were used as probes and labeled by the oligo-primer method with [³²P]dCTP (8). For each hybridization the probe was added at a concentration of 3 × 10⁶ to 6 × 10⁶ cpm/ml of hybridization buffer.

Preparation of cytoplasmic RNA. Total cytoplasmic RNA was prepared as described by Chomczynski and Sacchi (7) from MKT-1 cells, chicken kidney lymphoma induced by MDV, CEF infected with the CVI-988 strain, and mock-infected chicken kidney. Poly(A)⁺ RNA was isolated from total cytoplasmic RNA by oligo(dT)-cellulose chromatography (2).

Northern (RNA) blot hybridization. Northern blot hybridizations were done as described by Bradley et al. (3). Poly(A)⁺ RNA was denatured with 1 M glyoxal in aqueous 50% dimethyl sulfoxide as described by McMaster and Carmichael (18). Poly(A)⁺ RNA was electrophoretically separated on 1.0% agarose gel (10 µg of tissue RNA and 7.5 µg of cell RNA per slot), transferred to nylon membranes, and hybridized at 45°C overnight in 10 ml of hybridization buffer (0.6 M NaCl, 0.2 M Tris hydrochloride [pH 8.0], 0.02

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FIG. 1. Schematic representation of the MDV genome. It consists of a long unique sequence flanked by a terminal repeat (TRL) and an internal inverted repeat (IRL) and a short unique (US) sequence flanked by an internal (IRS) and an inverted terminal (TRS) repeat region. The long unique sequence is subdivided into UL1 and UL2. The *Bam*HI linkage map is shown. Solid bars indicate the region where MDV-specific transcripts were detected.

M EDTA [pH 8.0], 0.5% sodium dodecyl sulfate, 0.1% sodium pyrophosphate, 50% formamide). After hybridization, the membranes were washed twice with $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate–0.1% sodium pyrophosphate for 5 min at room temperature, followed by three washings with $0.1\times$ SSC–0.1% sodium dodecyl sulfate–0.1% sodium pyrophosphate for 10 min at 55°C . The dried gels were autoradiographed at room temperature or at -80°C on Kodak X-Omat AR X-ray film with intensifying screens. Chicken rRNAs of 28S (4.0 kb) and 18S (1.67 kb) were used as size markers.

To determine the sensitivity of our Northern blot procedure, various amounts of purified rabbit globin mRNA (BRL, Gaithersburg, Md.), ranging from 0.5 pg to 5 ng, were electrophoretically separated on 1.0% agarose gels and transferred to nylon membranes. Hybridizations were performed as described above. Rabbit globin cDNA was used as a probe and added at a concentration of 3×10^6 cpm/ml of hybridization buffer.

RESULTS

Regions encoding MDV-specific transcripts. In previous studies we cloned a *Bam*HI library of the MDV genome comprising approximately 95% of the genome. In this study we hybridized labeled MDV DNA fragments from each of the clones to the electrophoretically separated poly(A)⁺ RNA extracted from MDV-induced kidney lymphoma and from MKT-1 cells. The salient feature of the results is the detection of major bands containing MDV RNAs in extracts of both kidney lymphoma and MKT-1 cells. These transcripts were homologous to five *Bam*HI fragments (D, H, I₂, L, and A) comprising the terminal repeats flanking the long unique sequence (TRL and IRL) and the short component (IRS). Consistent with the hypothesis that all transcripts contained in kidney lymphoma and in MKT-1 cells are

derived from these fragments, no MDV-specific transcripts of the remaining 22 *Bam*HI fragments were detected in extracts of kidney lymphoma or in MKT-1 cells (Fig. 1). The results of negative hybridizations are not presented. Based on the results of Northern blot hybridizations with rabbit globin mRNA, we estimate that the detection level of Northern blot hybridization is less than one copy per cell, since we could detect 1 pg of rabbit globin mRNA (data not shown). If each cell produces one copy of a certain species of mRNA (1.0 kb), then 7.5 μg of poly(A)⁺ RNA applied to the gel should contain 48 pg of that specific mRNA, assuming that one cell contains 5 pg of RNA (1) and that 5% of total RNA is poly(A)⁺ RNA. We routinely obtained 150 to 200 μg of poly(A)⁺ RNA from 10^9 cells, compared with the expected value of 250 μg . On the basis of the sensitivity of our assays, we conclude that, with the exceptions of portions of the *Bam*HI A and D fragments, most of the unique sequence of the L component (UL), accounting for approximately 80% of the total MDV genome, was not transcribed or otherwise produced levels of transcripts too small to be detected.

Identification of MDV specific transcripts homologous to the terminal repeats flanking the L component (TRL and IRL) contained in the BamHI D and H fragments. *Bam*HI fragments D and H span the junction between the inverted repeats (TRL and IRL) and the long unique sequences (UL), and therefore they share the homologous repeat sequences. As previously reported, digestion of *Bam*HI-D with *Bgl*II yields four subfragments, designated in the order of their mapping as I, II, III, and IV. Similarly, digestion of *Bam*HI-H with *Eco*RI and *Sma*I yields four subfragments designated as I, II, III, and IV (Fig. 2C and 3C). Our studies indicate that three RNA transcripts of 3.8, 2.5, and 1.6 kb are homologous to the UL region of *Bam*HI-D subfragment III (*Bam*HI-D-), whereas no RNAs homologous to the UL region of *Bam*HI-H-I and -II were detected in either kidney lymphoma or in MKT-1 cells.

FIG. 2. MDV-specific transcripts in virus-induced kidney lymphoma and in the virus-nonproducing lymphoblastoid cell line MKT-1 are encoded by the D fragment of the *Bam*HI library. The sizes of the RNAs are shown in kilobases. Poly(A)⁺ RNA was also isolated from CEF infected with the MDV lytic strain CVI-988 (A). Northern blot hybridization was performed with subfragment probes, which together span the entire *Bam*HI D fragment. Lanes I through IV represent hybridization with probes I through IV, respectively. Restriction enzyme sites are shown. The sizes of the subfragments are as follows: I, 2.3 kb; II, 1.0 kb; III, 3.9 kb; and IV, 4.8 kb (panels B and C). Each lane has a different exposure time. (A) CVI, 2 h; KL, MKT-1, 3 days at room temperature. (B) I and II, 3 days; III, 4 days at room temperature; IV, 7 days at -80°C. (S) lanes represent short exposure of the indicated lanes: CVI(S), 30 min; KL(S) and MKT-1(S), 1 day at room temperature. The 4.0- and 1.7-kb bands indicate 28S and 18S rRNA, respectively.

FIG. 3. Kidney lymphoma and MKT-1 cells containing MDV-specific transcripts encoded by *Bam*HI-H and *Bam*HI-H subfragments. Lanes I through IV represent hybridization with probes I through IV, respectively. The sizes of the RNAs are shown in kilobases. The sizes of the subfragments are as follows: I, 1.4 kb; II, 0.8 kb; III, 0.95 kb; and IV, 2.3 kb (panel C). Each lane has a different exposure time. (A) CVI, 2 h; KL and MKT-1, 3 days at room temperature. (B) I and II, 7 days at -80°C ; III and IV, 3 days at room temperature. (S) lanes represent short exposure of the indicated lanes: CVI(S), 30 min; KL(S) and MKT-1(S), 1 day at room temperature. The 4.0- and 1.7-kb bands indicate 28S and 18S rRNA, respectively.

Seven RNA transcripts (3.8, 2.9, 2.4, 1.8, 1.6, 1.3, and 0.9 kb) were homologous to the inverted repeats (TRL and IRL) contained entirely in *Bam*HI-D-I and -II and *Bam*HI-H-III and -IV. The poly(A)⁺ RNA extracted from CEF lytically infected with CVI-988, an attenuated strain of MDV, contained six transcripts (3.8, 2.5, 2.0, 1.6, 1.3, and 0.4 kb) that were homologous to *Bam*HI-D. However, the 2.5- and 2.0-kb transcripts that were homologous to *Bam*HI-D (Fig. 2 and 3) did not hybridize with *Bam*HI-H, and therefore these transcripts most likely are derived from the U_L of *Bam*HI-D.

In earlier studies we reported on the amplification of a 132-bp repeat sequence contained within the repeat region of *Bam*HI-D-I and *Bam*HI-H-IV in a nonpathogenic strain of MDV (24). The amplification of this specific sequence correlated with the loss of tumorigenicity of MDV (10, 16, 21). Because of the heterogeneity in the number of repeats, hybridization of labeled probes of this region to electrophoretically separated digests of DNA sequences from attenuated viruses yields very broad bands or smears rather than the sharp bands that are characteristic of pathogenic strains.

Earlier studies also demonstrated that in lytic infection with the pathogenic MDV strain RBIB, transcripts of 3.8, 3.0, 1.8, and 1.7 kb and homologous to *Bam*HI-H were detected (4). The amplification of the 132-bp repeat sequence was not observed in MKT-1 cells (3). However, hybridization of electrophoretically separated poly(A)⁺ RNA from both kidney lymphoma and MKT-1 cells (Fig. 2B and 3B) with probes of this region yielded a smearlike hybridization pattern. The identically sized transcripts of 2.9, 2.4, 1.8, 1.6, 1.3, and 0.9 kb were also homologous to those of *Bam*HI-I₂ (Fig. 4). These data suggest that some transcripts encoded in the region of *Bam*HI-I₂ are identical to those found in the repeat region of *Bam*HI-D and -H, and they also suggest that some of these transcripts are transcribed only in tumors and latently infected cells.

Identification of transcripts homologous to *Bam*HI-I₂, -L, and -A fragments containing the sequences mapping to terminal repeats of the L component (TRL and IRL), the internal inverted repeats of the S components (IRS and TRS), and the unique sequences of the S component (US). Hybridization of labeled *Bam*HI-I₂ fragment to electrophoretically separated MKT-1 cell poly(A)⁺ RNA revealed nine species of RNA homologous to the probe. These transcripts were 4.0, 3.6, 2.9, 2.4, 1.8, 1.6, 1.3, 0.9, and 0.7 kb in size. Of these, only the 4.0- and 3.6-kb transcripts were not present in kidney lymphoma. Four RNA species (3.6, 2.9, 2.4, and 1.8 kb) were the most abundant transcripts in cells latently infected with MDV. Cells productively infected with CVI-988 contained a very abundant 1.6-kb transcript as well as less abundant transcripts of 3.6, 3.1, 2.7, 2.4, 0.9, and 0.7 kb that were homologous to this fragment (Fig. 4A and B). Digestion of *Bam*HI-I₂ with *Sst*II and *Xba*I yielded three subfragments (Fig. 4C). The four most abundant transcripts from MKT-1 cells were mainly homologous to subfragment III (Fig. 4B); these RNA transcripts, however, were not as abundant in lytically infected cells (data not shown).

Hybridization of electrophoretically separated MKT-1 RNAs with a *Bam*HI-L probe revealed a moderately abundant 0.6-kb RNA and faint levels of 2.4- and 0.8-kb species homologous to the probe. Both 0.8- and 0.6-kb transcripts were found in moderate amounts in kidney lymphoma. The cells lytically infected with CVI-988 contained abundant amounts of 0.6- and 0.9-kb transcripts and less abundant amounts of 2.4- and 1.6-kb transcripts homologous to *Bam*HI-L DNA, but an RNA transcript of 0.8 kb, corre-

sponding to that found in latently infected cells, could not be detected. Additional transcripts were detected in CVI-988-infected cells (Fig. 5).

Hybridization of labeled *Bam*HI-A probe to electrophoretically separated kidney lymphoma RNA and MKT-1 RNA identified five relatively low-level transcripts (8.4, 4.8, 2.5, 1.2, and 0.7 kb) homologous to the probe. These five transcripts, however, were present at pronounced levels in cells lytically infected with MDV (Fig. 6); these cells also contained transcripts of 7.5, 6.1, and 3.0 kb that were homologous to *Bam*HI-A probe.

The *Eco*RI digests of *Bam*HI-A yielded five subfragments (Fig. 6C). Of these, subfragments IV and V contained predominantly unique sequences of the S component (US). Analysis of electrophoretically separated kidney lymphoma and MKT-1 RNAs with these probes failed to reveal homologous transcripts (Fig. 6B). These results suggest that the transcripts homologous to the *Bam*HI-A fragment are derived mainly from the inverted repeats flanking the unique sequences of the S component (IRS and TRS).

DISCUSSION

Distribution on the physical map of the viral genome of the sequences homologous to the MDV transcripts accumulating in latently infected kidney lymphoma and MKT-1 cells. In this report we have presented the results of the hybridization of the DNA fragments comprising >95% of the MDV genome to electrophoretically separated transcripts derived from chicken kidney lymphoma caused by MDV and a cell line, MKT-1, derived from an MDV-induced tumor. The DNA probes that hybridized to RNAs contained in these cells were also hybridized to RNAs extracted from lytically infected cells. The salient features of our data are summarized in the form of a transcription map superimposed on a *Bam*HI linkage map of the GA strain of MDV, as determined by Fukuchi et al. (9) (Fig. 7). Relevant to these results are the following.

(i) Our studies indicate that in a kidney lymphoma induced by MDV and in the latently MDV-infected cell line MKT-1, respectively, there were 29 and 32 virus-specific transcripts differentiated by their sizes. The actual number of viral transcripts may differ from the above because some of the transcripts obtained from different fragments may be derived from identical transcriptional units but may differ in splicing. In this regard, detailed cDNA cloning studies are in progress. Preliminary data indicate that considerable splicing occurs between *Bam*HI-H and -I₂ as well as within *Bam*HI-I₂. Also, the study shows that both strands of viral DNA are transcribed and that some genes overlap in opposite directions.

(ii) The transcripts derived from kidney lymphoma and from the lymphoblastoid MKT-1 cell line were very similar. In one respect, this is not entirely unexpected, since the MKT-1 cell line was originally derived from a tumor induced by MDV. The similarity in the accumulating MDV transcripts suggests that the genetic complexity that is expressed in kidney lymphoma and in MKT-1 cells is not haphazard, but rather the orderly and possibly required expression of MDV genes for the maintenance of the MDV genome in the latent state. The variability of MDV transcripts accumulating in these cells has also been noted and should be emphasized. Thus the MKT-1 4.0- and 3.6-kb transcripts homologous to *Bam*HI-I₂ and the 2.4-kb transcript homologous to *Bam*HI-L were not detected in kidney lymphoma tissues.

FIG. 4. Kidney lymphoma and MKT-1 cells containing MDV-specific transcripts encoded by *Bam*HI-I₂ and *Bam*HI-I₂ subfragments. Lanes I through III represent hybridization with probes I through III, respectively. The sizes of the RNAs are shown in kilobases. The sizes of subfragments are as follows: I, 1.3 kb; II, 0.9 kb; III, 3.0 kb (panel C). Poly(A)⁺ RNA was also isolated from mock-infected chicken kidney and was then hybridized to *Bam*HI-I₂ (panel A). Each lane has a different exposure time. (A) CVI, 2 h; KL and MKT-1, 10 h. (B) I and II, 2 days; III, 1 day at room temperature. The (S) lane represents a short exposure of the indicated lane: CVI(S), 30 min at room temperature. The 4.0- and 1.7-kb bands indicate 28S and 18S rRNA, respectively.

These differences are of interest, particularly because MKT-1 cells were derived from a kidney lymphoma caused by MDV, and the 3.6-kb transcript homologous to *Bam*HI-I₂ was one of the most abundant RNA transcripts in MKT-1 cells. These results suggest that differences in the way in which the tumor cells are maintained may affect the accumulation of viral transcripts to a limited extent. However, more extensive studies will be required to define the significance and extent of similarities and differences in the MDV transcripts accumulating in kidney lymphoma and MKT-1 cells.

(iii) The striking feature of the transcriptional map presented in Fig. 7 is that all of the transcripts detected in the kidney lymphoma cells and in the MKT-1 cell line map in the inverted repeats flanking the long and short unique sequences and, to a much lesser extent, in the adjacent unique sequences. These results suggest that these regions of the genome encode sequences required for the maintenance of the latent state. Consistent with this conclusion, transcripts derived from lytically infected cells contain a set of transcripts homologous to this region which differs from that present in kidney lymphoma tissue and in transformed cells.

FIG. 5. Kidney lymphoma and MKT-1 cells containing MDV-specific transcripts encoded by the *Bam*HI-L fragment. The sizes of the RNAs are shown in kilobases. Each lane has a different exposure time: CVI, 2 h; KL and MKT-1, 3 days at room temperature. The (S) lane represents a short exposure of the indicated lane: CVI(S), 30 min at room temperature.

A more detailed mapping of the transcripts from latently and lytically infected cells will determine whether and to what extent those same genes are transcribed in both types of infection.

In a recent study of a nonproducing lymphoblastoid cell line, HP-1, Schat et al. (20) reported that the viral transcripts hybridized only to a restricted region located in the repeat sequences, as seen in kidney lymphoma tissue and MKT-1 cells. Maray et al. (17) also reported the transcription of the MDV genome in transformed cells. In their study, the transcription was not restricted to the repeat region but rather was distributed throughout the entire genome. This may be due to the presence of productive cells in the transformed cell population.

Function of transcripts accumulating in latently infected kidney lymphoma and MKT-1 cells. Previous studies of latently infected lymphoblastoid cells demonstrated that the latent MDV genome is maintained as an episome in multiple copies (25) and that viral DNA replication is tightly regulated under cellular DNA synthesis (13). Transcription of the MDV genome in MKT-1 cells and in tumor tissue was studied by analyzing the reassociation kinetics. In both types of cells the same level of viral DNA, ranging from 12 to 14%, is transcribed. Furthermore, competition experiments between tumor tissue and MKT-1 cells suggested that transcription of the MDV genome in MKT-1 cells is restricted to the same specific DNA sequence as in tumor tissue (22, 23). The studies involving hybridization of labeled DNA probes to electrophoretically separated RNAs confirmed the previous studies, and these experiments demonstrated that resident viral DNA in MDV-induced tumors is controlled in a manner similar to that observed for MKT-1 cells. The

studies also indicated that the expression of the MDV genome in MKT-1 cells closely reflects the expression of the MDV genome in tumor tissue. The MKT-1 cell line should prove quite useful for further studies of gene expression in the latent MDV genome.

Of the MDV transcripts detected in this study, those encoded by the *Bam*HI-*I*₂ region were of particular interest, especially in light of the identification by Tilloson et al. of the open reading frame in the middle of the fragment common to *Bam*HI-*I*₂ and *Eco*RI-Q within the long inverted regions of MDV DNA (J. K. Tilloson, H. J. Kung, and L. E. Lee, Abstr. 3rd Intl. Symp. on Marek's Disease, p. 128, 1988). Partial sequencing of the *Eco*RI-Q genomic region led to the finding of an amino acid sequence containing a sequence similar to that designated as a "leucine zipper." The leucine zipper has been found in DNA-binding proteins such as transcriptional activators and the *myc*, *fos*, and *jun* oncogenes (12, 15). The amino acid sequence deduced from the *Eco*RI-Q clone contains a similar basic sequence from amino acids 47 to 68, which is 50% homologous to the *jun* sequence in this region. In both kidney lymphoma and MKT-1 cells, the identically sized 2.9-, 2.4-, 1.8-, and 1.3-kb transcripts were detected in the *Bam*HI-*I*₂ region. These transcripts were strongly expressed in a region encoding the putative leucine zipper (*Bam*HI-*I*₂-III, Fig. 4). The transcripts encoded in *Bam*HI-*I*₂ are thought to play an important role in maintaining the latent state or tumorigenicity of MDV because (i) the most abundant transcripts in latently infected cells are encoded in *Bam*HI-*I*₂, (ii) it has been suggested that unique transcripts in latently infected cells are encoded in *Bam*HI-*I*₂, and (iii) one of these transcripts is thought to encode the DNA-binding protein of the leucine zipper.

For oncogenic DNA viruses in general, the immediate-early gene products are responsible for inducing tumors and for stable transformation, e.g., the T antigen of polyomavirus and simian virus 40. For tumor induction to occur, it is necessary that immediate-early gene products are expressed in nonpermissive or semipermissive cells to ensure cell viability. However, in the case of Epstein-Barr virus latency, latent genes and productive genes are clearly segregated. Whether the transcripts in *Bam*HI-A and the fragments discussed above do, indeed, encode the immediate-early gene products or specific latent gene products remains to be determined. We have found previously that this particular *Bam*HI-A fragment transformed NIH 3T3 mouse cells. Specifically, cells transfected with *Bam*HI-A fragment were morphologically altered and formed foci that caused tumors in nude mice.

Significance of the transcription and expression of viral gene functions in cells latently infected with MDV. The finding of numerous transcripts in kidney lymphoma tissue and in MKT-1 cells that harbor latent MDV is of interest from another point of view. On the basis of its biological behavior, MDV was originally classified as a member of the *Gammaherpesviridae* subfamily of the *Herpesviridae* family of viruses. However, certain features of MDV may be classified as having the character of *Alphaherpesviridae* rather than that of *Gammaherpesviridae*, because (i) the genome structure is that of the alpha family, in which the unique region is conserved, and (ii) its tropism is more toward fibroblasts (CEF) and epithelium (feather follicle epithelium) than toward lymphocytes. The unique features of MDV as a member of the gamma family consist of (i) lymphoma induction, (ii) establishment of lymphoblastoid cells carrying viral genomes, and (iii) restricted but active transcription of viral genes in latently infected cells, as observed in the

FIG. 6. Kidney lymphoma and MKT-1 cells containing MDV-specific transcripts encoded by *Bam*HI-A and *Bam*HI-A subfragments. In subfragments IV and V (panel C), RNA transcripts were not detected in kidney lymphoma or MKT-1 cells. Lanes I through V represent hybridization with probes I through V, respectively. The sizes of the RNAs are shown in kilobases. The sizes of the subfragments are as follows: I, 5.7 kb; II, 6.1 kb; III, 1.7 kb; IV, 2.8 kb; and V, 4.4 kb (panel C). Poly(A)⁺ RNA was isolated from mock-infected chicken kidney and then hybridized to *Bam*HI-A (panel A). Each lane has a different exposure time. (A) CVI, 2 h; KL and MKT-1, 4 days at room temperature; mock-infected chicken kidney, 3 days at -80°C . (B) I through III, 4 days at room temperature; IV and V, 7 days at -80°C . The (S) lane represents a short exposure of the indicated lane: KL(S) and MKT-1(S), 1 day at room temperature. The 4.0- and 1.7-kb bands indicate 28S and 18S rRNA, respectively.

present study. Among the members of the gamma subfamily are Epstein-Barr virus and related primate viruses and the herpes saimiri and ateles viruses. Characteristically, these viruses remain latent in lymphoblastoid cells, and in such

cells they express a subset of genes whose products in many instances have been identified and which function to maintain the genome in a latent state. In contrast, members of the *Alphaherpesviridae* subfamily, as exemplified by herpes

FIG. 7. Transcriptional map of the MDV genome in kidney lymphoma and MKT-1 cells. These data are based on the results of Northern blot hybridizations. RNA transcripts were positioned on the *Bam*HI restriction map of the genome of the MDV GA strain. A partial transcription map of the MDV genome of the CVI-988 strain is also presented.

simplex virus and varicella-zoster virus, remain latent in sensory ganglia. In cells harboring these viruses in a latent state, very few virus-specific transcripts have been detected, and those that have been detected are largely not polyadenylated and appear to accumulate in the nuclei (26). To date, no virus-specific protein products have been detected in these cells. Based on the present data, it is clear that at least some of the actively transcribed mRNA in latently infected cells is specific to the latent infection and is not found in productively infected cells. Thus the latent transcription aspect of MDV is more closely related to that of Epstein-Barr virus, in which genes for productive infection and for

latent infection are clearly segregated. Thus the studies presented in this report extend the similarity in the biological behavior of MDV to those of other members of the *Gamma-herpesviridae* family.

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