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RNA Transcripts of Marek's Disease Virus (MDV) Serotype-1 in Infected and Transformed Cells

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Abstract

RNA was isolated from two strains of Marek's disease virus (MDV-Z and MDV-B). The virus was grown in duck embryo fibroblasts (DEF) for 96 hr, 72 hr in the presence of phosphonoacetic acid (PAA) and 24 hr in the presence of cycloheximide added at the time of infection. With the use of DNA probes representing about 80% of the MDV genome, an extensive Northern blot analysis of the RNA was carried out. A similar analysis was done with RNA extracted from the MDV-transformed cell line MSB-1. This study revealed 42, 25 and 29 discrete viral RNA transcripts in MDV-Z and MDV-B-infected DEF and in the MSB-1 cell line, respectively, ranging in size from 0.8 to 13 kb. In MDV-Z-infected DEF, there were twelve late RNA species, two early and eight immediate-early viral transcripts. In MDV-B-infected DEF there were eleven late RNA species, two early and seven immediate-early viral transcripts. The RNA species were homologous for all the probes used except the *Bam*HI-G DNA fragment where no RNA transcripts were detected in the MSB-1 cell line. The RNA transcripts were used to produce a preliminary viral RNA map. Comparison of the location and sizes of the viral RNA transcripts in MDV-infected and MDV-transformed cells revealed several differences.

Introduction

Marek's disease virus (MDV), a member of the herpesvirus family, causes a malignant lymphoma of T-cell origin and lymphoproliferative infiltration in peripheral

nerves in chickens (1). Although little DNA homology was detected between the genomes of herpesvirus of turkeys (HVT) (serotype 3) and MDV (serotype 1) under stringent DNA-DNA annealing conditions (2–4), MDV-induced lymphomas can be prevented by vaccination of chickens with HVT (5). This is the first model system in which induction of tumors by a herpesvirus under natural conditions is prevented by vaccination with an apathogenic antigenically related virus.

Vaccination breaks in HVT-immunized chicken flocks in recent years were reported to be caused by the emergence of very virulent MDV (vvMDV) strains that were isolated from diseased chickens (6). Six local MDV serotype-1 strains were isolated by our group from naturally infected farm chickens during Marek's disease (MD) outbreaks in 1985 (T. Maray et al., to be published). These MDV isolates showed marked differences in their pathogenicity and virulence for chickens in the field and under experimental conditions. Five of these isolates (designated A, AB, B, G and M) were characterized as vvMDV strains. The sixth strain (designated MDV-Z), caused mainly leg paralysis in naturally infected chickens and differed from isolate MDV-B that caused tumors in naturally infected chickens. Unlike the vvMDV isolates (e.g., MDV-B), the DNA of MDV-Z was not detected in feather follicles of chickens infected with blood from naturally infected chickens. Modifications in the organization of the viral DNA were seen in the MDV-Z isolate when compared to DNA of the MDV GA strain, that served as a prototype. (T. Maray, M. Malkinson and Y. Becker, in preparation).

An abortive MDV infection of T cells in vivo leads to malignant transformation and results in a neoplastic disease. Akiyama et al. (7) first succeeded in establishing a Marek's disease lymphoma-derived T-lymphoblastoid cell line from infected chickens by culturing the cells at 41°C. Since then, more than 80 MDV-induced lymphoid cell lines have been produced (8–11). Evidence has been provided that the viral genome in the MD-lymphoid cell lines can be found integrated into cell DNA (as in the HPRS-1 cell line), or as both integrated and episomal forms (as in the MSB-1 cell line) or as mostly episomal DNA (as in the MKT-1 cell line) (12,13). A low level of expression of viral genes was found in these transformed lines (14).

MDV, HVT and other herpesviruses such as Epstein-Barr virus (EBV), H. saimiri, and H. ateles are grouped in the Herpesviridae subfamily, as Gammaherpesvirinae (15,16). Ono et al. (17) suggested that there are cross-reactive antigens between MDV and EBV. However, MDV and HVT have linear, double-stranded DNA with molecular weights of ca 120×10^6 and 103×10^6, respectively (18,19). Although MDV and HVT are biologically similar to EBV, H. saimiri, and H. ateles, their genomic structure and organization closely resemble herpes simplex virus type-1 (HSV-1) DNA that consists of two regions: a long unique (U_L) and a short unique (U_S) region, each flanked by two inverted repeat sequences, TR_L, IR_L, and TR_S, IR_S, respectively (20,21). Similar to other herpesviruses, the pattern of MDV gene expression could be considered to have a general "cascade" pattern that is divided into three distinct temporally regulated phases: immediate early (IE), early (E), and late (L).

Biochemical studies have shown that in MDV-infected cells, at least 35 MDV-specific polypeptides are produced (22). Six of these showed antigenic cross reactivity with HVT (23,24). The A antigen common to both MDV and HVT was recently identified and localized in the *Pvu*II-*Eco*RI sequence within the MDV *Bam*HI-B fragment in the U_L region (25). The complete sequence of antigen A was recently determined (Coussens P.M. and L.F. Velicer, 12th Inter national Herpesvirus Workshop, Pennsylvania, July 1987) as well as the expression of a 1.8 kb RNA transcript. Results from our laboratory showed that two mRNA species (2.1 and 3.7 kb) were detected by the MDV A antigen gene in MDV-and HVT-infected cells and in MDV-transformed cells (26). Recently, it was reported by Hirai et al. (27) that a 1.4 kb RNA species common to MDV serotype-1 and serotype-3 (HVT) is transcribed from the HVT *Bam*HI-J fragment. A specific RNA transcript of 2.0 kb was reported to be expressed from the *Bam*HI-H and *Bam*HI-D fragments in chicken embryo fibroblasts (CEF) infected with a tumorigenic strain of MDV (Hayashi H., Smith M., Tanaka A. and Nonoyama M., Eleventh International Herpesvirus Workshop, Leeds, July, 1986). However, no detailed mRNA map of MDV has been published.

In this study we investigated the patterns of RNA transcripts of MDV in duck embryo fibroblasts (DEF) infected with two local MDV serotype-1 isolates MDV-Z and MDV-B as well as in the MDV-transformed lymphoblastoid cell line MSB-1. The viral RNA species were detected by the Northern blot hybridization technique and were designated as IE, E, or L by using two metabolic inhibitors cyclohexamide and phosphonoacetic acid (PAA). The present study provided an overall analysis of MDV RNA transcripts in lytically infected cells and in an MDV-transformed cell line.

Materials and methods

Cells and viruses

The lymphoblastoid cell line MSB-1 (8) was cultured in RPMI-1640 medium containing 10% tryptose phosphate, 2% fetal calf serum and 8% calf serum at 41°C in a humidified atmosphere of 5% CO_2, at an initial concentration of 5×10^5 cells/ml. Subcultures were made every 2–3 days by dilution of the cells in RPMI-1640. Chicken embryo fibroblasts (CEF) and duck embryo fibroblasts (DEF) were prepared using procedures similar to those of Witter et al. (28).

HVT-strain F126 was provided as a lyophilized cell-associated vaccine preparation produced by Biological Laboratories, Teva (B.L.T.) (Jerusalem, Israel).

The MDV-Z and MDV-B isolates were obtained from MD-afflicted flocks located at different geographic regions in Israel. The MDV-B strain was isolated from 6-week-old broiler flocks. The MDV-Z strain was isolated from 6-week-old layer birds vaccinated at one day old with lyophilized HVT vaccine. The serotype determination of the two isolates was made by DNA-DNA hybridization and im-

munofluorescence of infected cell cultures with monoclonal antibodies to serotypes 1, 2, and 3 (kindly donated by Dr. L. Lee, East Lansing, Michigan; I. Davidson et al., to be published). Blood from MDV-Z and MDV-B naturally infected chickens was seeded on monolayers of DEF at 37°C. MDV serotype-1 isolates MDV-B and MDV-Z were passaged in DEF for 25 and 30 passages, respectively.

Infection of DEF with MDV isolates MDV-B and MDV-Z

DEF grown in Roux bottles were infected with MDV-B passage 24 and MDV-Z passage 29. The virus stocks were prepared by scraping of infected DEF from the culture bottles in a small volume of medium and freezing at −70°C. The stock virus preparations (as cell-associated virus) were thawed before addition to fresh DEF at a 1:9 dilution. After incubation for 12 hr, the medium was aspirated and fresh medium with or without metabolic inhibitors was added. By this method most of the cell debris in the virus stock was removed from the infected cultures. Since the virus is cell-associated, although the stock virus was diluted 1:9, contamination of the cultures with debris of infected cells from the inoculum cannot be ruled out. The infected cultures were harvested at 96 hrs p.i. At this time complete cytopathic effect was seen in the infected cultures. The medium was carefully removed and the infected cells were harvested by scraping with a rubber policeman, centrifuged, and the pellets were used for RNA extraction.

Use of metabolic inhibitors cycloheximide and phosphonoacetic acid (PAA)

For the detection of immediate-early (IE) RNA, DEF were infected as above and 100 μg/ml of cycloheximide was added at the time of infection. At 12 hr p.i., the medium and all debris of the virus inoculum were aspirated, fresh medium containing 100 μg/ml of cycloheximide was added and the infected cultures were reinoculated. At 24 hr p.i., the cultures were harvested as above and RNA was extracted. In the presence of cycloheximide, no cytopathic effects were seen in the infected cultures.

To study the early (E) viral RNA transcripts, DEF were infected as described above and 100 μg/ml of PAA was added at the time of infection. At 12 hr p.i., the medium containing cell debris from the inoculum was aspirated and fresh medium containing 100 μg/ml of PAA was added. The infected cultures were reincubated, harvested at 72 hr p.i., and used for extraction of RNA. Cytopathic effects were not seen in these cultures.

RNA extraction

RNA was extracted from uninfected DEF, MDV-Z-infected DEF, MDV-B-infected DEF, and from the MSB-1 cell line using the guanidinium thiocyanate pro-

cedure of Chirgwin et al. (29) as modified by Maniatis (30). The final RNA preparations were stored at −20°C in 70% ethanol and 0.2 M potassium acetate.

Labeling of the DNA probes

*Bam*HI DNA clones of MDV GA (kindly provided by Dr. M. Nonoyama (31)) were labeled by using a nick-translation reagent kit (Bethesda Research Laboratories) (30) with [^{32}P] dCTP (Amersham, U.K.) to a specific activity of approximately 1×10^8 cpm/μg. For each hybridization system, the probe was added at a concentration of 4×10^6 cpm/ml of hybridization buffer.

The *Bam*HI-DNA clones that were used in this study to detect viral RNA transcripts ranged in size from 1.5 kbp (*Bam*HI-P1) to 23.25 kbp (*Bam*HI-A) representing about 80% of the MDV genome. The MDV DNA genome restriction enzyme map was constructed by Fukuchi et al. (31). This map is included for orientation in Figs. 6 and 7.

Gel electrophoresis and Northern blot analysis

Each sample containing 10 μg of total RNA was electrophoresed in 1.5% agarose gels containing 18% formaldehyde and MOPS buffer (20 mM 3-[N-morpholiono] propanesulfonic acid, 5 mM sodium acetate and 1 mM EDTA) and transferred overnight onto nitrocellulose filters with 20 × SSC (1 × SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) by the Northern blot method (30). The filters were dried and baked at 80°C under vacuum conditions for 1.5–2 hr. Northern blot hybridizations were done under the conditions of 50% formamide and 5 × SSC at 42°C for 48 hr. The filters were washed, dried, and exposed to Agfa x-ray film with intensifying screen at −70°C from 30 min–6 hr for RNA extracted from infected, untreated cells. To detect viral RNA transcripts from cycloheximide or PAA-treated infected cells, the exposure time was increased to 12–24 hr.

The positions of the transcripts were determined relative to 28S (5 kb) and 18S (2 kb) ribosomal RNA.

Results

RNA transcripts from the short unique sequence (U_S) and its flanking repeat sequences (IR_S and TR_S)

The *Bam*HI-P1 and A DNA fragments cover most of the short unique (U_S) sequence and its internal inverted repeat (IR_S). Total RNA was extracted from DEF infected with each of the two Israeli MDV isolates (MDV-Z and MDV-B) and hybridized with the *Bam*HI-DNA fragments P1 and A.

Hybridization with the *Bam*HI-P1 DNA fragment (1.5 kbp), which is mapped to

the right-hand side of U_S, showed the presence of two RNA transcripts (2.6 and 0.8 kp) in the RNA extracted either from MDV-Z or MDV-B-infected cells (Fig. 1A).

The *Bam*HI-A DNA fragment (23.25 kbp in size), which maps to the left of *Bam*HI-P1 and covers most of the U_S and IR_S sequences in the viral genome, detected five RNA transcripts (11, 10, 6, 4.5, and 2.6 kb) in the RNA extracted from MDV-Z-infected DEF (Fig. 1B, lane 1). However, in MDV-B-infected DEF, only three viral RNA transcripts (11, 6, and 2.6 kb) were detected by the same probe, while the 10 and 4.5 kb RNA transcripts were not detectable (Fig. 1B, lane 2). A 2.6 kb RNA species was detected by both the *Bam*HI-P1 and *Bam*HI-A DNA fragments in the RNA extracted from DEF infected with MDV-Z or MDV-B. It is possible that the 2.6 kb RNA transcript arises from a gene that is located in the two DNA fragments *Bam*HI-A and P1.

A similar analysis was done with RNA extracted from the MDV-transformed MSB-1 cell line. Hybridization of MSB-1 RNA with the *Bam*HI-A DNA fragment revealed only three transcripts (11, 5, and 2.6 kb; Fig. 1B, lane 3). The 5 kb RNA transcript was unique to the transformed cell line only, while the 11 kb and 2.6 kb RNA species were detected in the RNA extracted from DEF lytically infected with MDV-Z or MDV-B isolates. The role of the 5 kb RNA transcript in the MDV-transformed cell is not yet known. It is of interest that viral RNA transcripts of 10 kb and 4.5 kb were expressed only in MDV-Z-infected DEF and not in MDV-B-infected cells.

RNA transcripts from the long unique (U_L) sequence and the flanking repeat sequences (TR_L and IR_L)

The unique long (U_L) and inverted repeat sequences (TR_L and IR_L) cover almost 80% of the MDV genome (31). The two inverted repeats of U_L contain the *Bam*HI-DNA fragments L, Q2, and 12. The *Bam*HI-H fragment (in IR_L) and *Bam*HI-D (in TR_L) are partially located in the repeat sequences and partially in the unique sequence. For this reason they share homologous sequences.

Viral RNA from TR_L and IR_L sequences

Hybridization of the *Bam*HI-L DNA fragment (3 kbp in size) to RNA extracts from DEF infected with MDV-Z showed the presence of four different species (4.5, 3, 1.9, and 1.2 kb; Fig. 2, lane 9).

Hybridization with the *Bam*HI-I2 (5.1 kbp) DNA fragment revealed two RNA transcripts (5 and 2.5 kb) in RNA extracted from DEF infected with either MDV-Z (Fig. 2, lane 7) or MDV-B (Table 1). In RNA extracted from the MSB-1 cell line, the *Bam*HI-I2 probe also detected two RNA transcripts (5 and 2.5 kb; Fig. 4, panel 3) that have the same molecular size as those detected in the RNA from DEF infected with the two MDV strains.

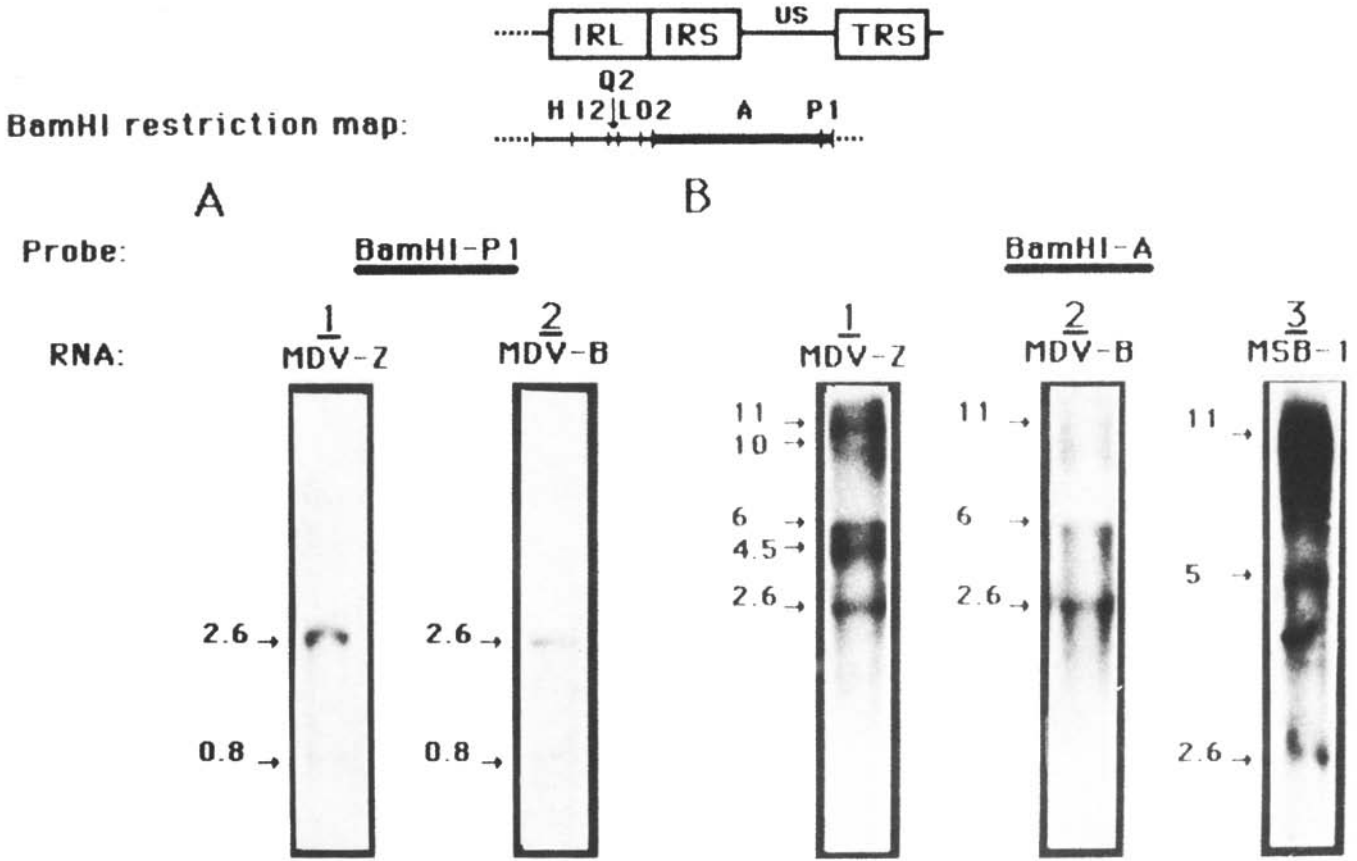


Fig. 1. RNA was extracted from MDV-Z-infected DEF, MDV-B-infected DEF and from the MDV-transformed MSB-1 cell line using the guanidinum thyocyanate procedure. The RNA was electrophoresed on 1.5% agarose gels containing 18% formaldehyde, transferred to nitrocellulose filters by the Northern blot method and hybridized to ^{32}P-labeled MDV-*Bam*HI recombinant clones of the GA strain. The probes were labeled using the nick translation method to a specific activity of approximately 1×10^8 cpm/μg. Northern blot hybridization was done under conditions of 50% formamide, 5 × SSC, at 42°C, for 48 hr with 4×10^6 cpm/ml of hybridization buffer. The filters were exposed O.N at −70°C for 6 hr. The analysis of hybridization with the *Bam*HI-A and *Bam*HI-P1 probes is presented.

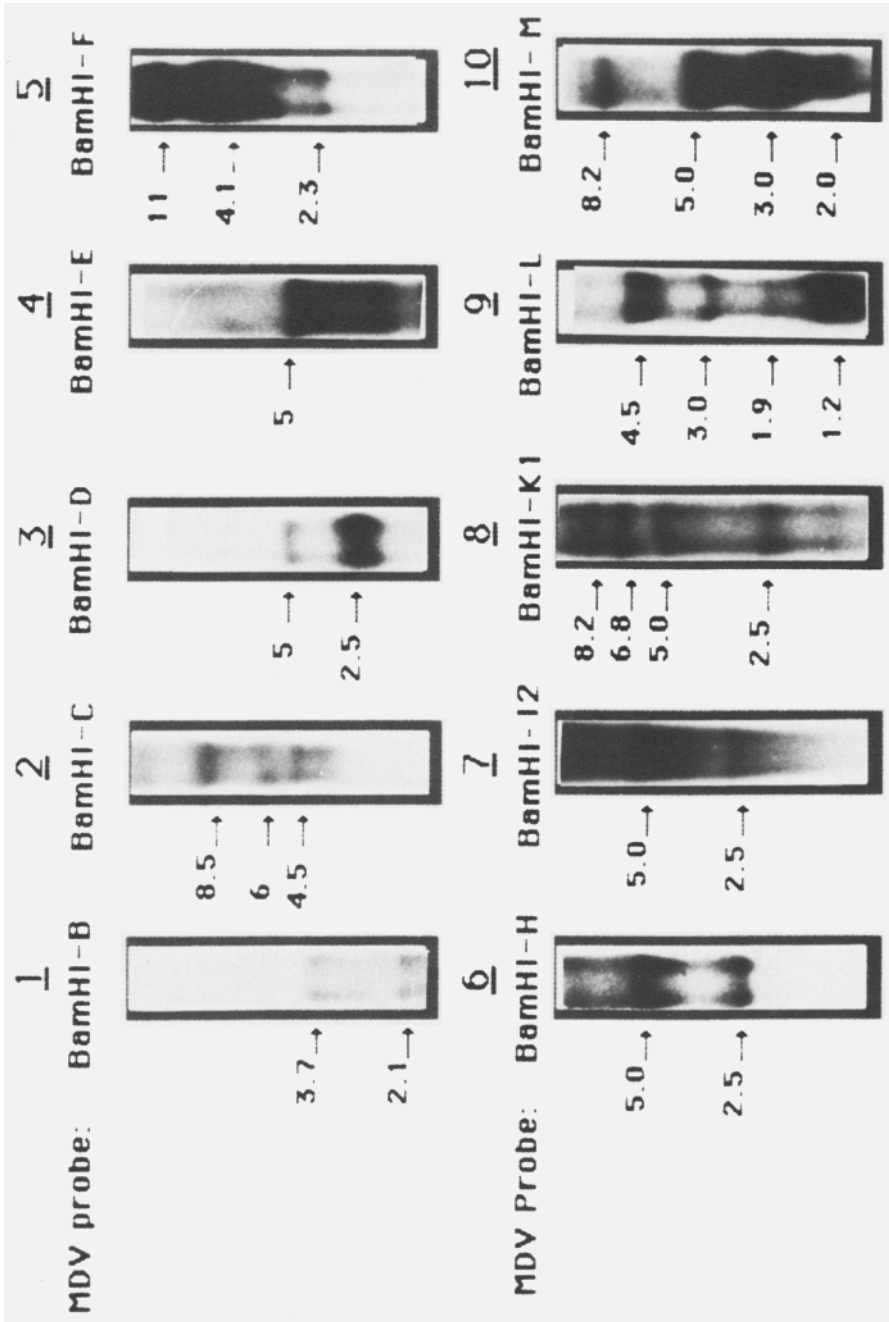


Fig. 2. Northern blot analysis of RNA extracts from MDV-Z infected DEF. The RNA extraction and the Northern analysis were done as described in the legend to Fig. 1.

Table 1. Sizes of RNA transcripts detected by MDV BamHI-DNA fragments in total RNA extracted from MDV-Z-infected DEF, MDV-B-infected DEF, and the MSB-1 cell line

		RNA EXTRACTED FROM		
		DEF infected with		
Probe	Size kbp	MDV-Z (kb)	MDV-B (kb)	MDV transformed MSB-1 cell line (kb)
*Bam*HI-A	23.25	11, 10, 6 4.5, 2.6, 0.9	11, 6, 2.6 0.9	11, 5, 2.6
*Bam*HI-B	18.3	3.7, 2.1	3.7, 2.1	10, 5, 3.7 3.4, 2.7, 2.1
*Bam*HI-C	14.85	8.5, 6, 4.5	8.5, 6, 2.5	8.5, 6
*Bam*HI-D	11.4	5, 2.5	5, 2.5	5, 3
*Bam*HI-E	9.6	5	4.5, 1.7	5
*Bam*HI-F	8.85	11, 4.1, 2.	4.1, 2.3	10, 3.8, 1.5
*Bam*HI-G	7.05	5	4.5	N.D*
*Bam*HI-H	5.4	5, 2.5	5, 2.5	5, 3, 2.5
*Bam*HI-I2	5.1	5, 2.5	5, 2.5	5, 2.5
*Bam*HI-J	4.35	13, 9.4 5.4, 4.5	13, 4.5 2.5	10, 5.6
*Bam*HI-K1	3.6	8.2, 6.8 5, 2.5	—**	6.2, 4.5 2.5, 1.2
*Bam*HI-K2	3.6	7.8, 6 3.5	—	5.6
*Bam*HI-L	3	4.5, 3 1.9, 1.2	—	—
*Bam*HI-M	2.25	8.2, 5 3, 2	—	—
*Bam*HI-P1	1.5	2.6, 0.8	2.6, 0.8	—

*N.D = not detected
**— = not done

The *Bam*HI-D (11.5 kb) and *Bam*HI-H (5.4 kb) fragments detected two RNA species (5 and 2.5 kb; Fig. 2, lanes 3 and 6, respectively) in RNA extracted from MDV-Z infected DEF. The same RNA species were detected in RNA extracted from MDV-B-infected DEF (Fig. 3, lane 4; Table 1). Since the *Bam*HI-H and the *Bam*HI-D DNA fragments share homologous sequences due to their presence in the IR_L and TR_L sequences respectively, it is possible that at least two viral RNA species are encoded from these homologous sequences. In MSB-1 cell RNA, the *Bam*HI-D probe detected two RNA transcripts (5 and 3 kb; Fig. 4, panel 1). The *Bam*HI-H DNA probe detected three RNA transcripts (5, 3, and 2.5 kb) in MSB-1 (Fig. 4, panel 2). The 3 kb RNA was the most abundant RNA species in MSB-1 cells.

Since the 3 kb RNA transcript was detected in MSB-1 by the *Bam*HI-H and *Bam*HI-D fragments but not by the DNA fragments *Bam*HI-I2 or *Bam*HI-K1 that

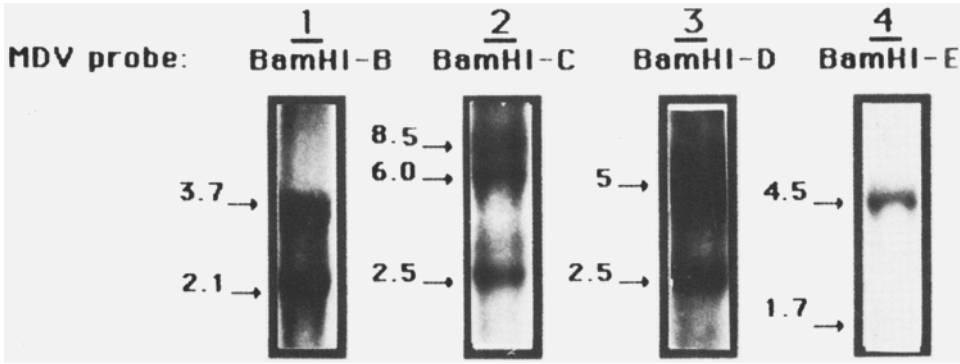


Fig. 3. Northern blot analysis of RNA extracts from MDV-B-infected DEF. The RNA extraction and the Northern blot analysis were done as described in the legend to Fig. 1.

map to the right and to the left of *Bam*HI-H, respectively, it is possible that this gene is located in the homologous sequence of *Bam*HI-H and *Bam*HI-D.

Viral RNA from the U_L sequence

The *Bam*HI-K1 (3.6 kbp) DNA fragment (located on the right-hand side of the U_L sequence next to the *Bam*HI-H fragment) detected four RNA transcripts (8.2, 6.8, 5, and 2.5 kb; Fig. 2 lane 8) in RNA from DEF infected with the MDV-Z strain. In MSB-1 RNA, four RNA transcripts (6.2, 4.5, 2.5, and 1.2 kb) were detected by the *Bam*HI-K1 DNA probe (Fig. 4, panel 4). It is of interest that only the 2.5 kb RNA transcript was detected in RNA prepared from MDV-Z-infected DEF and in MSB-1 cells. Since the *Bam*HI-K1 probe is 3.6 kbp in size, the RNA transcripts longer than 2.5 kb must be transcribed from the adjacent DNA sequences of the genome.

Fragment *Bam*HI-M (2.25 kbp in size), which maps on the left side of the *Bam*HI-K1 fragment, detected four RNA transcripts (8.2, 5, 3, and 2 kb; Fig. 2, lane 10) in the RNA extracted from MDV-Z-infected DEF. The two RNA transcripts of 8.2 kb and the 5 kb transcripts were also detected by *Bam*HI-K1. These results suggest that these two transcripts might originate from a gene that spans the *Bam*HI-K1 and *Bam*HI-M DNA sequences.

Hybridization of the *Bam*HI-B DNA fragment (18.3 kbp) with RNA from DEF infected with MDV-Z (Fig. 2, lane 1) and MDV-B (Fig. 3, lane 1), detected two RNA species of the same size (3.7 and 2.1 kb). In MSB-1 RNA, the *Bam*HI-B DNA fragment detected six RNA transcripts (10, 5, 3.7, 3.4, 2.7, and 2.1 kb; Table 1). The two RNA transcripts (3.7 and 2.1 kb) detected by the *Bam*HI-B DNA fragment were reported to be coded by the gene for antigen A that is located in *Bam*HI-B (26). These results indicate that, in addition to the two RNA transcripts arising

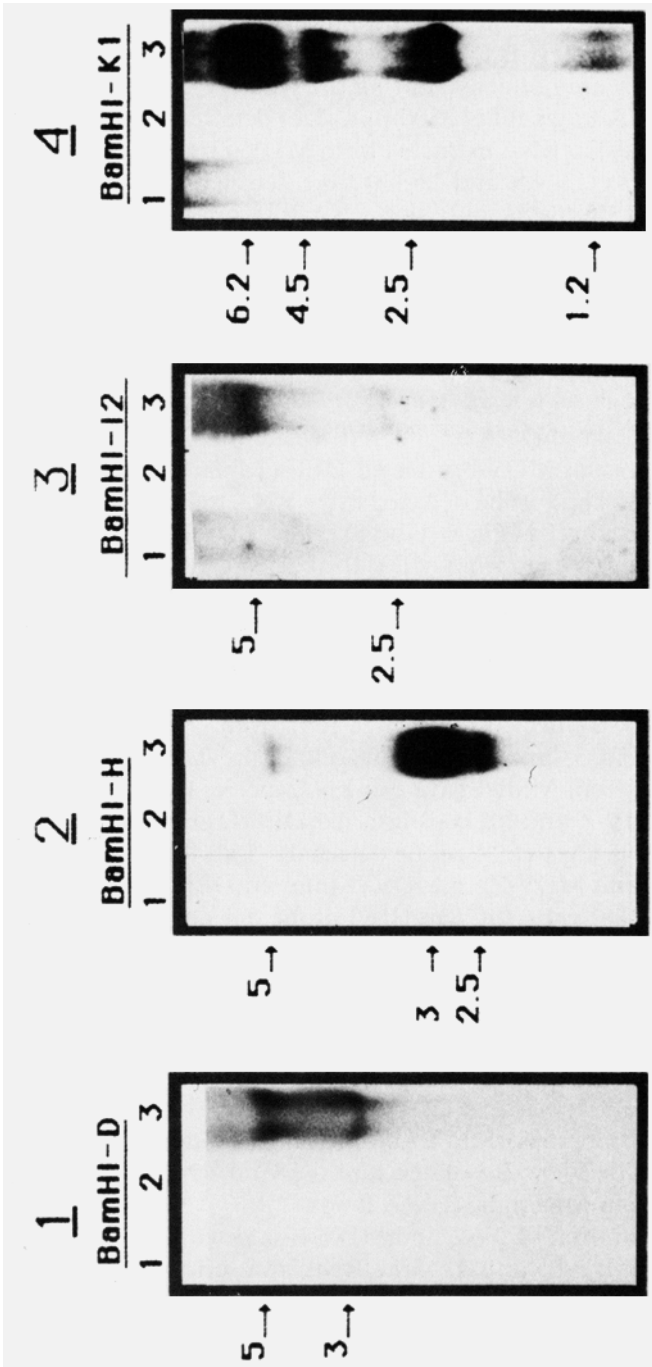


Fig. 4. Northern blot analysis of RNA extracts from the MSB-1 cell. The RNA extraction and the Northern blot analysis were done as described in the legend to Fig. 1. Lanes: 1) NaOH-treated RNA, 2) RNA treated with RNase, and 3) Untreated RNA.

from the DNA sequence at the A antigen gene, four additional genes were transcribed from the *Bam*HI-B DNA sequence in the MDV-transformed MSB-1 cell line. These genes are not active during the lytic cycle of the virus in DEF.

The *Bam*HI-J DNA fragment (4.35 kbp in size) detected four RNA transcripts (13, 9.4, 5.4, and 4.5 kb) in RNA extracted from MDV-Z-infected DEF, while only three RNA transcripts (13, 4.5, and 2.5 kb) were detected in RNA from MDV-B-infected DEF. In MSB-1 cells, only two RNA transcripts (10 and 5.4 kb) were detected (Table 1).

Using *Bam*HI-E (9.6 kbp in size) as a probe, a 5 kb RNA transcript was detected in RNA extracted from MDV-Z-infected DEF (Fig. 2, lane 4), while two RNA transcripts (4.5 and 1.7 kb) were detected in the RNA from MDV-B-infected DEF (Fig. 3, lane 4). In the MSB-1 cell line, one RNA species (5 kb) was detected by this probe (Table 1).

The *Bam*HI-K2 (3.6 kbp) DNA fragment detected three RNA transcripts (7.8, 6, and 3.5 kb) in RNA from MDV-Z infected DEF (Table 1).

The *Bam*HI-F (8.85 kbp) probe detected three RNA transcripts (11, 4.1, and 2.3 kb) in MDV-Z-infected cells (Fig. 2, lane 5). The 11 kb RNA transcript was not detected in the RNA extracted from MDV-B-infected cells using the same probe. Different results were seen in MSB-1 RNA, in which the *Bam*HI-F DNA fragment detected three RNA transcripts (10, 3.8, and 1.5 kb; Table 1).

The *Bam*HI-C (14.85 kbp) DNA fragment detected three RNA transcripts (8.5, 6, and 4.5 kb) in RNA extracted from MDV-Z-infected DEF (Fig. 2, lane 2). In RNA extracted from MDV-B-infected DEF three RNA transcripts (8.5, 6, and 2.5 kb) were also detected (Fig. 3, lane 2). Hybridization of the *Bam*HI-C DNA fragment with RNA extracted from MSB-1 gave two RNA species (8.5 and 6 kb) that were also detected in MDV-Z and MDV-B-infected DEF (Table 1).

The *Bam*HI-G (7.05 kbp) DNA probe detected a 5 kb RNA transcript in RNA from cells infected with MDV-Z. In MDV-B-infected DEF, a 4.5 kb RNA species was detected. In MSB-1 cells, the *Bam*HI-G probe did not detect any viral RNA transcripts (Table 1).

Characterization of MDV RNA transcripts as immediate-early (IE), early (E), and late (L) species

To characterize the properties of IE, E and L viral RNA transcripts, DEF were infected with MDV-Z or MDV-B isolates, and the viral RNA transcripts were extracted as described in Materials and Methods.

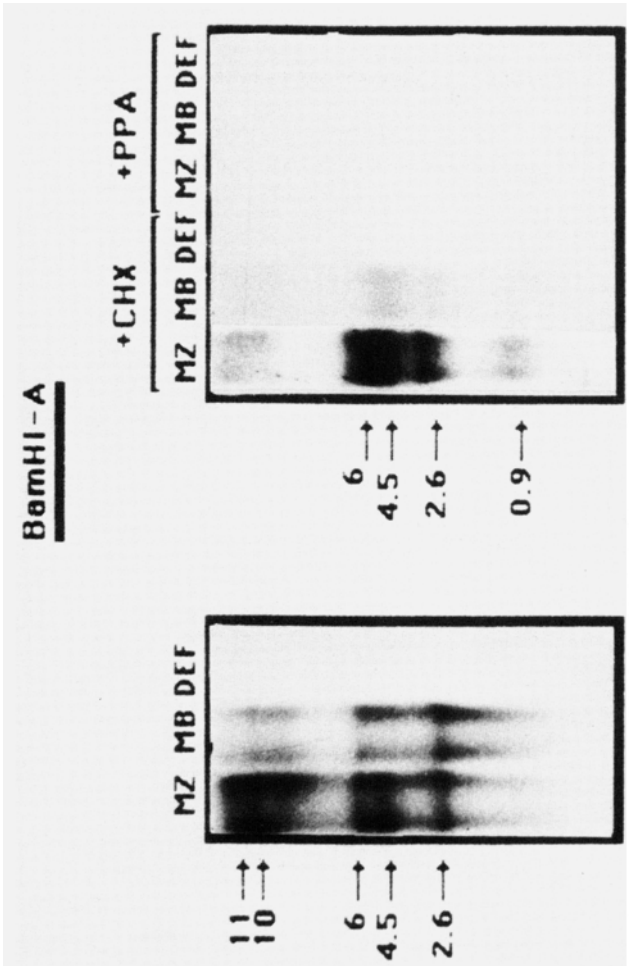
RNA was extracted from MDV-Z and MDV-B-infected DEF that were left untreated (to produce IE, E, and L viral RNA transcripts) or incubated with cycloheximide (to allow accumulation of IE RNA transcripts) or phosphonoacetic acid (to allow accumulation of IE and E RNA transcripts), and hybridized to ^{32}P-labeled MDV DNA *Bam*HI-A, B, C, D, E, F, G, H, and P3 fragments (Table 2). The results of hybridization with the *Bam*HI-A (Fig. 5A) and *Bam*HI-F (Fig. 5B) DNA

Table 2. Determination of IE (α), E (β) and L (γ) RNA transcripts in RNA extracted from DEF infected with MDV-Z or MDV-B

RNA IN MDV		Z STRAIN				B STRAIN			
Fra.	M.W(kbp) DNA	RNA kb	*CHX 0.1mg/ml	**PAA 0.1mg/ml	α	RNA kb	*CHX 0.1mg/ml	**PAA 0.1mg/ml	α
A	23.25	11	—	—	γ	11	—	—	γ
		10	—	—	γ	—	—	—	
		6	6	—	α	6	6	—	α
		4.5	4.5	—	α	—	—	—	
		2.6	2.6	—	α	2.6	2.6	—	α
		—	0.9	—	α	—	0.9	—	α
B	18.3	3.7	—	—	γ	3.7	—	—	γ
		2.1	—	—	γ	2.1	—	—	γ
C	14.85	8.5	—	—	γ	8.5	—	—	γ
		6	—	—	γ	6	—	—	γ
		4.5	—	—	γ	2.5	—	—	γ
D	11.4	5	5	5	β	5	5	5	β
		2.5	2.5	2.5	β	2.5	2.5	2.5	β
E	9.6	5	—	—	γ	4.5	—	—	γ
						1.7	—	—	
F	8.85	11	—	—	γ	—	—	—	γ
		4.1	4.1	4.1	α	4.1	4.1	4.1	α
		2.3	2.3	2.3	α	2.3	2.3	2.3	α
G	7.05	5	—	—	γ	4.5	—	—	γ
H	5.4	5	5	—	α	5	?	—	α
		2.5	2.5	—	α	2.5	?	—	α
P1	1.5	2.6	—	—	γ	2.6	—	—	γ
		0.8	—	—	γ	0.8	—	—	γ

*CHX = cycloheximide
**PAA = phosphonoacetic acid
— = not detected

fragments are presented. In RNA from MDV-Z infected cells treated with cycloheximide, four RNA transcripts (6, 4.5, 2.6, and 0.9 kb) were detected by the *Bam*HI-A DNA fragment (Fig. 5A). The 6, 2.6, and 0.9 kb RNA transcripts were detected at a low level in RNA extracted from cells infected with the MDV-B isolate. These RNA species were present in low amounts since they were detected after exposure of the filters for 12–24 hr while RNA transcripts in infected untreated cells were more abundant and shorter exposures for 0.5–6 hr were required. In PAA-treated cells, under conditions that inhibit MDV DNA replication, no transcripts were detected by *Bam*HI-A (Fig. 5A). All the transcripts isolated were classified as IE gene products. The 11 kb and 10 kb RNA transcripts and the 11 kb transcript detected in MDV-Z and MDV-B-infected, untreated DEF respectively, were not detected in the presence of cycloheximide or PAA. Therefore, they were classified as L viral RNA transcripts. It is of interest that the 6, 4.5, and 2.6 kb and



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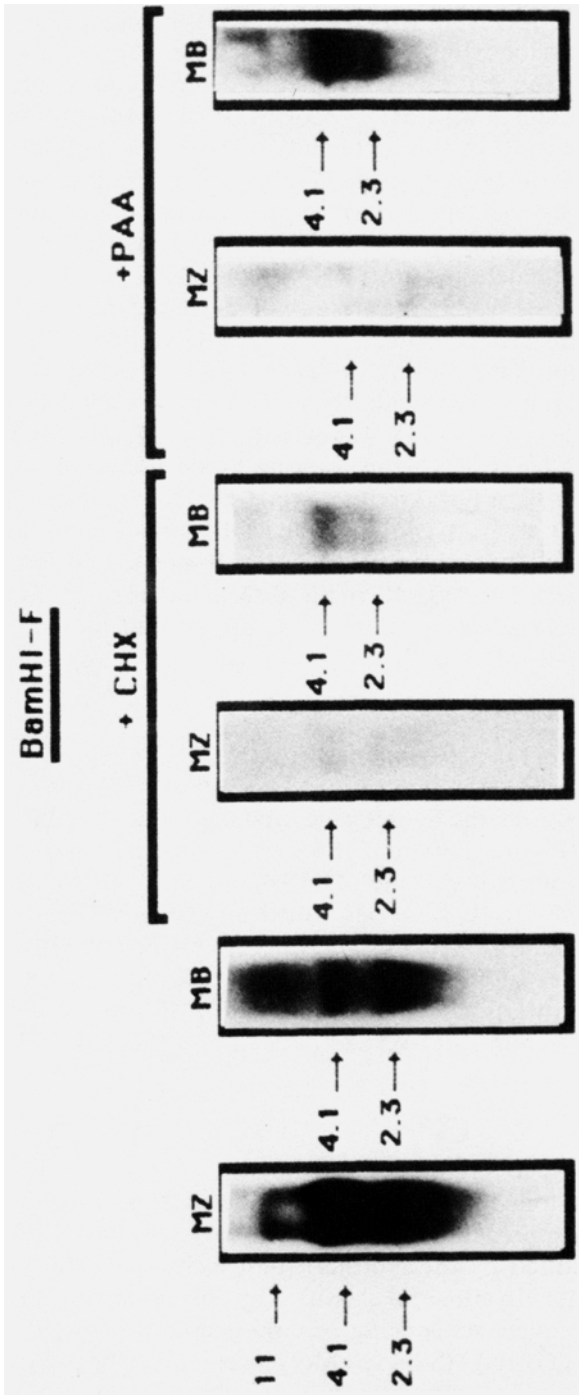

Fig. 5. Total RNA was extracted from MDV-Z-infected DEF, MDV-B-infected DEF and from uninfected DEF in the presence of 100 μg/ml of cycloheximide, 100 μg/ml of phosphonoacetic acid or from untreated cells (as described in Fig. 1). Northern blot hybridizations were done with the *Bam*HI-A clone (A), and with the *Bam*HI-F clone (B). The filters were exposed for 6 hr to detect MDV RNA transcripts from infected, untreated cultures. (A and B: untreated samples). To detect viral IE and E transcripts in the RNA from cycloheximide or PAA-treated infected cultures the filters were exposed for 12–24 hr (A and B: cycloheximide and PAA treated samples). DEF = RNA from uninfected duck embryo fibroblasts (DEF); MZ = RNA from MDV-Z-infected DEF; MB = RNA from MDV-B-infected DEF.

the 6 and 2.6 kb RNA transcripts from MDV-Z and MDV-B respectively were detected in the presence of cycloheximide but not in the presence of PAA.

The *Bam*HI-B, C, E, G, and P1 DNA fragments detected no viral RNA transcripts in DEF infected either with MDV-Z or MDV-B in the presence of cycloheximide or PAA. The two RNA species (5 and 2.5 kb) detected in untreated DEF infected with MDV-Z or MDV-B isolates after hybridization with the *Bam*HI-D DNA fragment were also detected in the presence of cycloheximide or PAA (Table 2). These two transcripts were considered to be IE genes.

The *Bam*HI-F DNA fragment detected three RNA species (11, 4.1, and 2.3 kb) in RNA from DEF infected with MDV-Z in the absence of metabolic inhibitors (Fig. 5B). In RNA from MDV-Z-infected cells treated with cycloheximide or PAA, the 4.1 and 2.3 kb RNA transcripts were found. It is assumed that the last two RNA transcripts are either IE or E genes. The 11 kb RNA transcript was not detected in the presence of cycloheximide or PAA and it was classified as an L gene. In MDV-B-infected DEF, the 4.1 and 2.3 kb RNA species were found under all the conditions and they may be either IE or E transcripts. *Bam*H-H DNA fragment detected 5 and 2.5 kb RNA transcripts in MDV-Z-infected DEF under conditions of cycloheximide but not PAA. It is of interest that in MDV-B-infected DEF the two RNA species (5 and 2.5 kb) were detected only in the absence of the true inhibitors. These two transcripts were classified as IE genes assuming that they occur in cycloheximide-treated MDV-B-infected DEF at an undetectable level.

Preliminary transcription map of the MDV genome

Data from Tables 1 and 2 and from the figures were used to produce a preliminary viral RNA transcript map (Fig. 6). Different RNA transcripts detected in total RNA extracted from DEF infected with the MDV-Z and MDV-B isolates and from MDV-transformed MSB-1 cells were positioned on the *Bam*HI restriction enzyme map of the DNA genome of MDV GA strain as published by Fukuchi et al., (31). The DNA probes used in this study cover about 80% of the entire MDV genome map. The viral RNA transcripts were positioned in relation to the viral DNA fragments used for detection (Fig. 6).

Discussion

Studies on the characterization of herpesvirus genes were done in numerous laboratories using different approaches: a) herpes simplex virus type 1 (HSV-1) RNA transcripts were identified by the Northern blot technique. The mRNA species were isolated, translated in vitro and classified by polyclonal antibodies to HSV-1 proteins (32). b) Complete sequencing of viral genomes [e.g., EBV (33), varicella-zoster virus (VZV) (34) and HSV-1 (D. McGeoch et al., to be published)] that allows identification of open reading frames of putative genes was done.

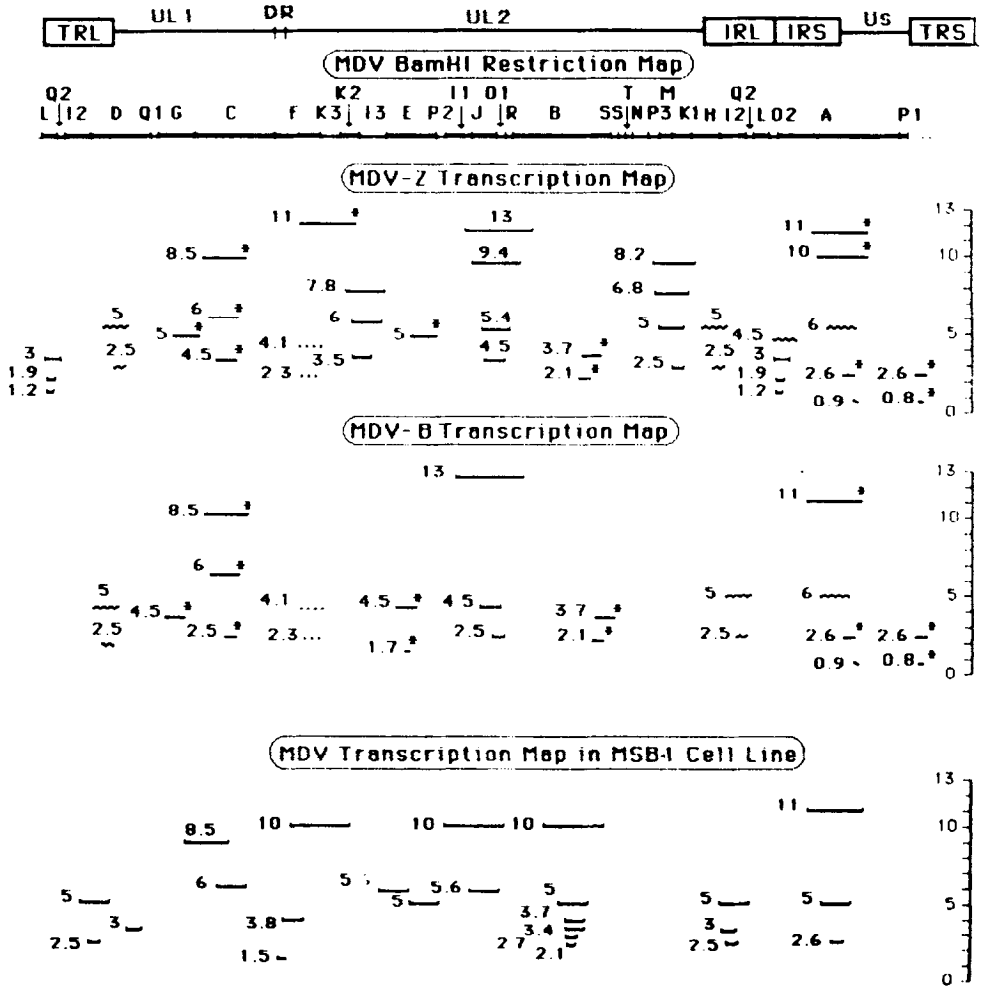

Fig. 6. Transcription map of the MDV genome based on data from Tables 1 and 2. Immediate-early = ~~~~, Early = · · · ·, Late = _____* and Undetermined = _____.

c) Cloned viral DNA fragments were transcribed and translated under in vitro conditions and the protein products were identified with specific antibodies (35). Although MDV is of vital importance in agriculture, understanding of the molecular processes of this virus lags far behind the knowledge of HSV-1. To help in the understanding of MDV RNA gene function, we concentrated on the identification of MDV RNA transcripts in lytically infected cells and compared them to the viral RNA transcripts detectable in MDV-transformed cells.

Use of the Northern blot hybridization procedure allowed us to identify approximately 42, 25, and 29 viral RNA transcripts in cells infected with serotype-1 MDV-Z and MDV-B and in the MDV-transformed cell line MSB-1, respectively (Table

1). Furthermore, we have partly characterized the IE (α), E (β) and L (γ) transcripts in MDV-Z and in MDV-B by using the two metabolic inhibitors, cycloheximide to inhibit protein synthesis and phosphonoacetic acid to inhibit viral DNA replication. Treatment of infected cells with the metabolic inhibitors prevented the spread of virus infection and the development of CPE limiting the infection to the cells that were infected by the stock virus. Indeed, the amount of viral RNA detected by the DNA probes was quite low since exposure of the filter for 12–24 hr was required. Since large fragments of viral DNA were used as probes, several RNA transcripts were detected by each fragment. It is not possible to distinguish between multiple RNA species of the same size mapping in the same DNA fragments. The exact position and orientation of each RNA transcript was not defined, thus only approximate locations of the RNA transcripts were determined. Further studies on the fine mapping of viral mRNA are needed.

Large RNA transcripts (> 10) were detected in most of the gels after a long period of autoradiography. It is assumed that some of these transcripts are artifacts of high molecular weight RNA complexes or precursors of smaller RNA transcripts. The presence of spliced genes cannot be predicted from our analysis, but it is reasonable to assume that splicing is rare during the expression of MDV genes by analogy to HSV-1 (36).

In the present study, we used 15 MDV *Bam*HI DNA fragments ranging from 23.25 kbp (*Bam*HI-A)–1.5 kbp (*Bam*HI-P1) and detected 42 virus-specific RNA species in DEF infected with the MDV-Z strain. It was noted (Table 1) that most, but not all, of the RNA transcripts detected in MDV-B-infected cells were identical to those detected in MDV-Z RNA. This result indicated that the two MDV isolates, which differ in their pathogenicity for chickens, function differently in the lytic cycle. Further studies will identify the variation in gene usage between these two MDV isolates. It is of interest that many of the RNA transcripts produced in lytically infected DEF were also found in the transformed MSB-1 cells. This might be explained by spontaneous reactivation of MDV replication in the transformed cells. Since a small percentage of the cells produce viral antigens as measured by staining the cells with anti-MDV antibodies that detect MDV A and B antigens, it is not clear which viral RNA species are specific for the transformed state of the tumor cells.

The results documented in Table 2 provide information on RNA species that are the products of the immediate-early genes. These genes are localized in the DNA of the *Bam*HI-A fragment (6, 4.5, 2.6, 0.9 kb RNA transcripts) and in *Bam*HI-D and -H fragments (5 and 2.5 kb RNA transcripts) suggesting at least 6 IE genes. There is a similar number of IE genes in HSV-1 infected cells. RNA transcripts from some early and late genes were also detected. Among the late genes, two RNA species (3.7 and 2.1 kb) were found to hybridize with the gene for the viral A antigen (26). The late RNA species coding for viral B antigen are being studied. The early genes that code for viral enzymes are under-represented in the analysis of RNA transcripts.

Fig. 7 provides comparative data that relate the positions in the viral glyco-

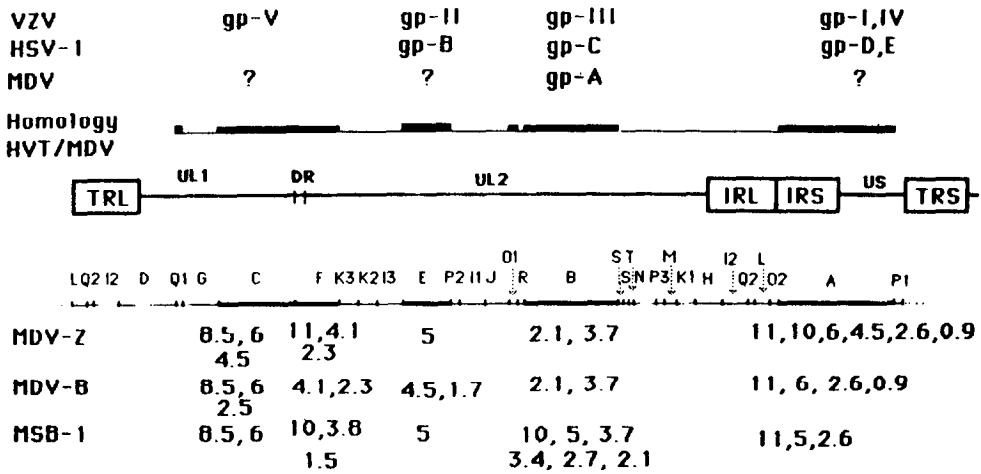

Fig. 7. Location of the VZV and HSV-1 glycoproteins as compared with the predicted location of MDV glycoproteins.

proteins of the genomes of VZV (33), HSV-1 (33,37,38) and MDV. The relative positions of HSV-1 and VZV glycoproteins are the same and they correspond with the MDV/HVT homologous sequences (39). In Fig. 7 we indicate which RNA species were detected by MDV *Bam*HI-DNA fragments C, F, E, B, and A, and which correspond to the glycoprotein genes. The position of the MDV A antigen in the *Bam*HI B fragment was identified (25). Thus, MDV *Bam*HI DNA fragment A should contain genes for two viral glycoproteins and MDV *Bam*HI DNA fragments C and F, E, and possibly G, should contain the two additional genes for glycoproteins; one of them should be the gene for the MDV B antigen.

The map of MDV RNA transcripts presents an initial attempt to obtain a general view of MDV gene function in lytically infected and transformed cells. On the basis of the preliminary RNA transcript map it will be possible to identify individual functional genes in each fragment of the viral DNA either by direct nucleotide sequencing or by in vitro transcription/translation techniques.

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