Latency of Herpesvirus of Turkey and Marek's Disease Virus Genomes in a Chicken T-Lymphoblastoid Cell Line

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SUMMARY

The properties of latent herpesvirus of turkey (HVT) and Marek's disease virus (MDV) genomes have been studied in virus-non-producer MDCC-BO1(T) cells, a T-lymphoblastoid cell line derived from spleen cells of an HVT-vaccinated chicken. The numbers of the two virus genomes in BO1(T) cells remained stable at 1.6 to 1.8 HVT genome equivalents/cell and 3.4 to 3.8 MDV genome equivalents/cell throughout a number of passages and were not decreased by the presence of phosphonoacetic acid in the culture. When the culture temperature of the MDV-producer MDCC-MSB1 cell line was shifted from 41 to 37 °C, the cells cultured at 37 °C contained about five times as many virus genomes as those cultured at 41 °C. In contrast, the numbers of the two virus genomes in BO1(T) cells were not increased by culture at 37 °C. RNA extracted from BO1(T) whole cells and from the polyribosomal fraction hybridized to both MDV and HVT DNAs, indicating the expression of both latent virus genomes. Digestion of cell nuclei with micrococcal nuclease revealed that both latent HVT and MDV genomes possess a nucleosomal structure. Closed circular MDV DNA was demonstrated in BO1(T) by isopycnic centrifugation of DNA in ethidium bromide-CsCl gradients.

INTRODUCTION

Marek's disease virus (MDV) is the aetiologic agent of Marek's disease (MD), a highly contagious malignant lymphoma of chickens. A herpesvirus of turkey (HVT) has been successfully used as MD vaccine. Although HVT protects chickens from MD, the vaccination does not prevent persistent infection by MDV. A chicken T-lymphoblastoid cell line, MDCC-BO1(T), has been established from the spleen cells of an apparently healthy chicken vaccinated with HVT, and naturally infected with MDV after vaccination (Kitamoto et al., 1980). Neither herpesvirus nor C-type particles were detected in BO1(T) cells by electron microscopy. More than 95 % of the cells had MD tumour-associated surface antigen (MATSA). However, no virus internal antigens or membrane antigens could be demonstrated by immunofluorescence tests using chicken anti-HVT and MDV sera. The virus could be rescued from BO1(T) cells by co-cultivation with chick embryo fibroblasts (CEF). The DNA of the rescued virus was characterized as HVT DNA by its restriction endonuclease HindIII cleavage pattern. DNA–DNA reassociation kinetics showed that the BO1(T) cells contained HVT and also MDV genomes. The origin of MDV genomes in this cell line is not clear. Non-specific pathogen-free (SPF) chickens had been kept with these experimental chickens for a while after the first HVT vaccination. Thus, the MDV genomes detected in BO1(T) cells...
might have been derived from these non-SPF chickens. This is the first cell line that has been established from an HVT-vaccinated chicken. Therefore, it is important to study the properties of this cell line to understand the nature of the latent HVT and MDV genomes. Recently, it has been reported that MDV DNA can exist as an episome (Tanaka et al., 1978) or in an integrated state (Kaschka-Dierich et al., 1979).

In the present work we examined the latencies of the HVT and MDV genomes in BO1(T) cells and the transcription of the two virus DNAs. Evidence for the nucleosomal structure of latent virus DNA and for the circularity of latent MDV DNA is presented.

METHODS

Virus and cells. The C2 strain (Kato et al., 1970) of MDV and the O1 strain (Ono et al., 1974) of HVT were propagated in primary CEF. Infection of cell-associated virus was carried out as described previously (Hirai et al., 1980).

BO1(T) (Kitamoto et al., 1980), MDCC-MSB1 (Akiyama & Kato, 1974) and MSB-U v-1 cells were maintained at 41 °C in RPMI 1640 medium supplemented with 10% foetal calf serum. MSB-UV-1 cells are a cell line cloned after brief exposure of MSB1 under u.v. light.

Extraction of DNA from virions. MDV and HVT DNAs were extracted from the Nonidet P40 (NP40)-treated virions and further purified by sedimentation in neutral glycerol gradients as described previously (Hirai et al., 1979).

Extraction of RNA from whole cells and polyribosomal fractions. The polyribosomal fraction was prepared as described by Brawerman et al. (1972). RNA was extracted from cell pellets and polyribosomal pellets by sequential extractions with neutral and alkaline tris-buffered phenol as described by Tanaka et al. (1977).

Nick translation. Virus DNA was labelled with $^3$H-TTP in vitro as described previously (Hirai et al., 1979). The specific activities of MDV and HVT $^3$H-DNAs were $5.6 \times 10^6$ and $5.2 \times 10^6$ ct/min/µg respectively. The DNAs were used for DNA–DNA reassociation and DNA–RNA hybridization kinetics.

DNA–DNA reassociation kinetics. Conditions for DNA–DNA reassociation kinetics were previously described (Hirai et al., 1979). A 0.02 µg amount of $^3$H-HVT or MDV DNA ($5.6 \times 10^6$ and $5.2 \times 10^6$ ct/min/µg respectively) was mixed with sonicated virus or cell DNA in 0.5 ml 0.3 M-NaOH, boiled for 15 min and neutralized with HCl. Then 5 M-NaCl was added to a final concentration of 2 M in 1 ml of the mixture and samples were incubated at 67 °C. At suitable times during incubation, 0.1 ml samples were removed and stored at -20 °C. Later they were assayed by single-strand-specific nuclease digestion (nuclease S1; Seikagaku Kogyo Co., Tokyo, Japan) to determine the amount of reassociated $^3$H-labelled virus DNA. The following equation was used for analysis of the data: $(C_0/C)^{1/0.55} = 1 + kCt$, where C and C0 are the concentrations of single-stranded $^3$H-virus DNA at times t and t = 0 respectively, and k is the reassociation constant (Britten & Davidson, 1976). The mol. wt. of MDV DNA, HVT DNA and cell DNA were calculated as 110 x 10^6 (Hirai et al., 1979), 97 x 10^6 (K. Hirai, unpublished data) and 1.4 x 10^{12} (Sober, 1970) respectively.

DNA–RNA hybridization kinetics with RNA excess. $^3$H-virus DNA (20000 ct/min) was mixed with 0.6 to 5 mg RNA in 0.9 ml 0.002 M-EDTA pH 7.2. The mixture was then boiled for 10 min and quickly chilled and mixed with 0.1 ml 5 M-NaCl. Samples were incubated at 67 °C. At suitable times during incubation, samples of 0.1 to 2 ml were removed and stored at -20 °C. Later they were assayed by single-strand-specific nuclease digestion (nuclease S1) to determine the amount of hybridized virus DNA. Self-hybridization of $^3$H-virus DNA was measured in the presence of RNA extracted from uninfected CEF cells.
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The amount of self-hybridization was subtracted from the corresponding hybridization values. Hybridization was plotted as the percentage $^3$H-virus DNA hybridized versus Rot = mol nucleotide of RNA x s/l (Frenkel & Roizman, 1972).

**Preparation of nucleosomal fractions.** Cells were washed three times with phosphate-buffered saline and suspended in a solution of 0.34 M-sucrose, 0.06 M-KCl, 0.014 M-NaCl, 0.015 M-2-mercaptoethanol, 0.002 M-EDTA, 0.015 M-tris-HCl pH 7.4, containing 0.5% NP40. The suspension was gently mixed, centrifuged at 1000 g for 5 min, the pellet washed with the digestion medium (0.34 M-sucrose, 0.001 M-CaCl₂, 0.06 M-KCl, 0.015 M-NaCl, 0.015 M-2-mercaptoethanol, 0.015 M-tris-HCl pH 7.4) and resuspended in the digestion medium at a concentration of 2 x 10⁸ nuclei/ml. The nuclei were digested with micrococcal nuclease (Worthington Biochemical Co., Freehold, N.J., U.S.A.) at a concentration of 1 µg/ml at 37 °C for 10 min. The digestion was terminated by adding 0.2 M-EDTA to a final concentration of 0.05 M. The digested chromatin was then dialysed against 0.2 mM-EDTA overnight at 4 °C and centrifuged at 1000 g for 10 min. The supernatant was layered on to 48 ml of a linear gradient of 5 to 20% sucrose in 0.01 M-tris-HCl pH 9, containing 0.2 mM-EDTA and centrifuged in a Hitachi RPS 25-2 rotor for 24 h at 75 000 g and 2 °C. The gradients were fractionated from the bottom and the absorbance of each fraction was measured at 260 nm. The monomer, dimer and trimer fractions were pooled, dialysed against 0.2 mM-EDTA and the DNA extracted.

**Agarose gel electrophoresis.** About 60 µl of the nucleosomal DNA was subjected to electrophoresis on a 2% horizontal agarose gel in E buffer (0.12 M-tris-HCl pH 7.2, 0.003 M-EDTA, 0.06 M-sodium acetate). Gels were stained with ethidium bromide (EtBr, 0.5 µg/ml) for 30 min and photographed under u.v. light. The HindIII fragments of PM2 DNA were used as reference species (Bracket al., 1976) in estimating the size of the nucleosomal DNA.

**Detection of MDV and SV40 DNA sequences by DNA–RNA hybridization.** Single-stranded DNA immobilized on membrane filters was hybridized with MDV and SV40 $^3$H-complementary (c)RNA made in vitro by Escherichia coli DNA-dependent RNA polymerase as described previously (Hirai, 1977). In control experiments, SV40 and MDV $^3$H-cRNA did not hybridize to DNAs from uninfected CV-1 cells and from chick blood cells and the amount of radioactivity bound to the membranes was the background level obtained without immobilized DNA (200 to 250 ct/min).

**EtBr–CsCl equilibrium centrifugation.** BO1(T) cells were lysed in 1% sodium dodecyl sulphate, 0.1 M-NaCl, 0.1 M-tris-HCl pH 9, and incubated with 1 mg/ml Pronase at 37 °C overnight. The lysate was gently mixed with an equal volume of phenol and centrifuged at 5000 g for 10 min. The upper layer was dialysed against 0.2 mm-EDTA for 2 days. Then 8 ml of the DNA solution in 0.01 M-tris-HCl pH 8, 0.01 M-EDTA was mixed with 0.6 ml of 4 mg/ml EtBr and 9 g solid CsCl and centrifuged in an Hitachi RPS55T for 48 h at 105 000 g and 18 °C. The DNA extracted in the same manner from SV40-infected CV-1 cells was separately centrifuged and each fraction was hybridized with SV40 $^3$H-cRNA to detect SV40 DNA sequences (Hirai, 1977). The fractions of BO1(T) cell DNA corresponding to closed circular and open circular or linear SV40 DNA were pooled and tested for the presence of MDV DNA by DNA–RNA hybridization studies. The concentration of DNA in the gradient was kept at less than 3 µg/ml to reduce physical entanglement of closed circular DNA with viscous cell DNA.

**Immunofluorescence test.** The direct immunofluorescence test to detect virus-induced intracellular antigens was performed as described previously (Kitamoto et al., 1980).

**Phosphonoacetic acid (PAA).** PAA was prepared as described by Hirai & Watanabe (1976) and neutralized with NaOH. Disodium phosphonoacetate was dissolved at a concentration of 10 mg/ml in PBS and autoclaved.
Fig. 1. DNA–DNA reassociation kinetics of 3H-MDV and HVT DNA with DNA of BOI(T) and MSB1 cells cultured with and without PAA (100 μg/ml). A 0.02 μg amount of 3H-HVT or -MDV DNA (5.6 × 10^6 and 5.2 × 10^6 ct/min/μg respectively) was mixed with virus or cell DNA. DNA–DNA reassociation kinetics were examined as described in Methods. (a) 3H-MDV DNA was mixed with the following DNAs in 1 ml; O, 2 mg chicken blood (CB) cell DNA; ●, 0.785 μg cold MDV DNA (5 genomes/cell) and 2 mg CB cell DNA; △, 2 mg BOI(T) cell DNA; ▲, 2 mg DNA from BOI(T) cells cultured with PAA; ●, 0.4 mg MSB1 cell DNA; □, 0.4 mg DNA from MSB1 cells cultured with PAA. (b) 3H-HVT DNA was mixed with the following DNAs in 1 ml; O, 2 mg CB cell DNA; ●, 0.71 μg HVT DNA (5 genomes/cell) and 2 mg CB cell DNA; △, 2 mg BOI(T) cell DNA; ▲, 2 mg DNA from BOI(T) cells cultured with PAA.

RESULTS

Effect of PAA on the number of virus genomes in BOI(T) cells

The BOI(T) cell line has been shown to contain 1.6 HVT genome and 3.5 MDV genome equivalents/cell (Kitamoto et al., 1980). The cells were maintained at a split ratio of 1:10 for each passage. Under these conditions, after over 80 passages the amounts of virus DNA remained remarkably stable at 1.6 to 1.8 HVT genome and 3.4 to 3.8 MDV genome equivalents/cell (data not shown).

To determine whether the two virus genomes were concentrated in a few virus-producer cells or existed as latent DNA, we cultured the cells in the presence of PAA (100 μg/ml), since PAA has been shown to inhibit the replication of MDV DNA (Nazerian & Lee, 1976) and HVT DNA (K. Ikuta et al., unpublished data). As shown in Fig. 1 (a), on culture in the presence of PAA, the virus genome equivalents/cell of partial producer (0.1 to 1%) MSB1 cells decreased from 56 to 11.5, as found by Nazerian & Lee (1976), whereas the number of HVT and MDV genomes in BOI(T) cells did not decrease (Fig. 1a, b). The growth rate of MSB1 and BOI(T) cells was not decreased at a concentration of 100 μg/ml PAA. The results indicate that both HVT and MDV genomes in BOI(T) cells exist as latent DNA and that
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Table 1. Effect of culture temperature on the amount of virus genomes in MSB1 and BO1(T) cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Virus genome</th>
<th>No PAA 41 °C</th>
<th>PAA 41 °C</th>
<th>No PAA 37 °C</th>
<th>PAA 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSB1</td>
<td>MDV</td>
<td>53.1</td>
<td>11.3</td>
<td>247.0</td>
<td>13.5</td>
</tr>
<tr>
<td>MSB-UV-1</td>
<td>MDV</td>
<td>4.5</td>
<td>3.9</td>
<td>24.8</td>
<td>4.1</td>
</tr>
<tr>
<td>BO1(T)</td>
<td>MDV</td>
<td>3.9</td>
<td>3.8</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>HVT</td>
<td>1.7</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

replication could be regulated under host cell control. The reassociation of 3H-virus DNA and BO1(T) cell DNA proceeded linearly without a break (Fig. 1), suggesting that most of the virus DNA sequences are present in BO1(T) cells.

Effect of culture temperature on the number of virus genomes in BO1(T) cells

MSB1 cells were usually grown at 41 °C, and at this temperature 0.1 to 1% of the cells were MDV antigen-positive. When the cells were cultured at 37 °C, growth became slower while virus antigen-positive cells gradually increased to about 10% at 5 days after the temperature shift (data not shown).

The amounts of MDV genomes in MSB1 cells at 41 and 37 °C were examined by DNA–DNA reassociation kinetics (Table 1). Results showed that the cells cultured at 37 °C contained about five times more MDV genome equivalents/cell than cells cultured at 41 °C. Even in the subclone of MSB1, MSB-UV-1, which contained only 4.5 genome equivalents/cell at 41 °C, the amount of MDV genomes increased to 24.8 virus genome equivalents/cell on culture for 5 days at 37 °C. However, when the MSB1 and MSB-UV-1 cells were cultured at 37 °C in the presence of PAA after cultivation in the presence of PAA at 41 °C, it was found that the amounts of virus genomes were almost the same at both temperatures. These results suggest that the increased numbers of virus genomes in the two cells after the temperature shift is due to increased numbers of the cells replicating MDV DNA and that the temperature shift does not affect the amount of latent virus DNA. There was no significant difference in the amounts of MDV and HVT genomes in BO1(T) cells at 41 and 37 °C with or without PAA (Table 1), and further cultivation of BO1(T) cells at 37 °C did not change the amount of virus DNA. Therefore, the majority of cells in the BO1(T) cells seem to be non-producers of the viruses.

Expression of latent virus genomes

More than 95% of the BO1(T) cells had MD tumour-associated surface antigen (MATSA) on their cell surface, and no virus intracellular or membrane antigens could be demonstrated in them by the immunofluorescence test (Kitamoto et al., 1980). However, it is not known whether MATSA is a virus-gene product. Therefore, the presence of virus mRNA in BO1(T) cells was examined by DNA–RNA hybridization kinetics (Fig. 2).

Examination of the kinetics of hybridization of 3H-HVT DNA with RNA extracted from CEF whole cells infected with HVT or from the polyribosomal fraction, showed that more than 45% transcription of the HVT genome occurred, suggesting that, if the virus DNA is asymmetrically transcribed, most of the HVT genome may be transcribed (Fig. 2a). Polyribosomal RNA also hybridized to the same extent with HVT DNA, indicating that most of the virus RNA sequences detected in the whole cells are transferred to the polyribosomes.
Fig. 2. DNA–RNA hybridization kinetics of $^3$H-HVT and $^3$H-MDV DNA with RNAs extracted from HVT- and MDV-infected CEF and BO1(T) cells. $^3$H-HVT or $^3$H-MDV DNA (20,000 cts/min) was mixed with RNA. DNA–RNA hybridization kinetics were examined as described in Methods. $^3$H-virus DNA was mixed with the following RNAs in 1 ml. (a) $^3$H-HVT DNA with 0.6 mg total RNA from HVT-infected CEF; $^3$H-HVT DNA with 0.6 mg polyribosomal RNA. (b) $^3$H-MDV DNA with 0.6 mg total RNA from MDV-infected CEF; $^3$H-MDV DNA with 0.6 mg polyribosomal RNA. (c) $^3$H-HVT DNA with 5 mg total RNA from BO1(T) cells; $^3$H-HVT DNA with 2.5 mg polyribosomal RNA. (d) $^3$H-MDV DNA with 5 mg total RNA from BO1(T) cells; $^3$H-MDV DNA with polyribosomal RNA.

The extent of MDV genome transcription in CEF whole cells infected with MDV was also more than 45% (Fig. 2b; Silver et al., 1979).

RNA extracted from BO1(T) whole cells cultured at 41 °C hybridized to 11 to 13% of HVT DNA and 7 to 8% of MDV DNA (Fig. 2c, d). Therefore, the most abundant RNA species are complementary to only a fraction of the HVT and MDV genomes. In BO1(T) cells, the RNA found in the polyribosomal fraction also hybridized to 6 to 7% of HVT DNA and 3 to 4% of MDV DNA (Fig. 2c, d). Therefore, the result indicates that, at least a portion of the HVT and MDV transcripts in the whole BO1(T) cells are transferred to the polyribosomes.

Next, BO1(T) cells were treated with 25 μg/ml iododeoxyuridine (IdUrd) for 3 days and then assayed by the immunofluorescence test for the induction of virus antigens. Results showed that approx. 0.01% of the cells gave a positive reaction for HVT intracellular antigens (data not shown). Therefore, the expression of latent HVT genomes may be activated to a certain extent.

**Nucleosomal structure of latent virus DNA in BO1(T) cells**

To see whether the latent virus genomes have the nucleosomal structure, which is a repeated subunit structure of chromatin, we digested the nuclei of BO1(T) cells with micrococcal nuclease and separated the digested chromatin subunits by centrifugation in neutral sucrose gradients (Fig. 3a). For identification of the fractions of subunits, the DNA extracted from each fraction in Fig. 3a was subjected to electrophoresis on a 2% agarose gel (Fig. 3b). The size of the nucleosomal DNA was determined from a semi-logarithmic plot of the number of base pairs of HindIII-digested fragments of PM2 DNA (Brack et al., 1976).
As shown in Fig. 3(b), the repeating lengths of the nucleosomal DNA in BO1(T) cells were indistinguishable from the 207 to 210 base pairs reported for chicken erythrocytes (Kornberg, 1977) and fraction II and III were identified as monomer and dimer of chromatin subunits respectively. If the virus DNA is folded into nucleosomes as host chromatin there should be no difference between the ratio of virus DNA to cellular DNA in DNAs of digested and undigested BO1(T) cell nuclei. Analysis by DNA–DNA reassociation kinetics of 2 mg samples of DNAs extracted from nuclei before and after micrococcal nuclease digestion and from the monomer and dimer of the chromatin subunits (Fig. 4a, b), showed that the reassociation with $^3$H-HVT and -MDV DNA in all the DNA preparations proceeded linearly and at similar velocities without a break. These results indicate that the latent HVT and MDV genomes are both folded into the nucleosomal structure and that there may not be any great difference between the repeating lengths of virus and cellular DNAs. However, the reassociation of $^3$H-MDV DNA with DNA of undigested MSB1 cell nuclei was about 2 to 4 times faster than with DNAs of digested nuclei and chromatin subunits (data not shown). Therefore, some of the virus DNA in the producer cell nuclei is not protected from digestion. For interpretation of data based on DNA–DNA reassociation kinetics, the lengths of DNA in DNAs of digested and undigested nuclei should be about the same. Therefore, DNA...
Fig. 4. DNA–DNA reassociation kinetics of $^3$H-MDV and -HVT DNA with nucleosomal DNA of BO1(T) cell nuclei. (a) $^3$H-MDV DNA (0.02 μg) was mixed with the following DNAs in 1 ml: ○, 2 mg CB cell DNA; ●, 2 mg total DNA from undigested BO1(T) cell nuclei; △, 2 mg DNA from BO1(T) cell nuclei digested with micrococcal nuclease; ▲, 2 mg DNA from nucleosome monomers of BO1(T) cell nuclei in fraction II of Fig. 3 (a); □, 2 mg DNA from nucleosome dimers in fraction III of Fig. 3 (a). (b) $^3$H-HVT DNA (0.02 μg) was mixed with the same DNAs as shown in (a). The symbols for each DNA are the same as in (a).

Fig. 5. EtBr–CsCl equilibrium centrifugation of BO1(T) cell DNA. DNA was extracted from SV40-infected CV-1 (a) and BO1(T) cells (b) and centrifuged in EtBr–CsCl density gradients as described in Methods. The DNAs of each fraction in (a) were hybridized with $^3$H-SV40 cRNA to detect the virus DNA sequences. DNAs of fractions I to VIII shown in (b) were tested for the presence of MDV DNA sequences by DNA–RNA hybridization. The arrows in (b) indicate the positions as buoyant densities of closed circular (CC) and open circular (OC), or linear (L), virus DNA.

Preparations were degraded to 200 to 300 nucleotides in length by boiling in 0.3 M-NaOH for 15 min before reassociation with probe virus DNA (data not shown). We believe that the size range of DNA fragments in this experiment was adequate to allow interpretation of the kinetic data.

Circular structure of latent MDV DNA

It has been reported that most latent MDV genomes in a virus-non-producer cell line established from an MD tumour exist as circular episomal DNA (Tanaka et al., 1978). For examination of the circularity of virus DNA in BO1(T) cells, the DNA extracted from the cells was centrifuged in EtBr–CsCl density gradients (Fig. 5). For location of the regions of closed circular and open circular or linear DNA in the gradient, DNA extracted in the same manner from SV40-infected CV-1 cells was centrifuged in the EtBr–CsCl gradient and hybridized with $^3$H-cRNA (Fig. 5 a). Fraction III shown in Fig. 5 (b) was found to correspond to closed circular DNA by correlation of buoyant densities. Measurement of the DNA content of each fraction by the diphenylamine reaction showed that cellular DNA was located in fraction VI. Fractions from each portion were pooled from eight centrifuge tubes
and the MDV DNA sequences in the gradient of BO1(T) cell DNA were detected by DNA–RNA hybridization. As shown in Fig. 5(b), more than 20% of the MDV DNA in the cells was detected in the form of closed circular DNA molecules. However, no closed circular form of HVT DNA was detected. This could be due either to the low virus DNA content or to the absence of closed circular HVT DNA molecules.

**DISCUSSION**

This investigation showed that both HVT and MDV genomes in BO1(T) cells have various properties of latent genomes. The numbers of virus genomes in BO1(T) cells remained stable throughout passage and were not decreased by the presence of PAA in the culture medium. Nazerian & Lee (1976) examined the effect of PAA on MDV producer MSB1 cells and found that it caused a decrease in the amount of MDV DNA, detected by DNA–RNA hybridization. Our quantitative analysis by DNA–DNA reassociation kinetics confirmed their results and showed that PAA inhibits productive MDV DNA synthesis but has no effect on the replication of latent MDV DNA. Since the growth rate of the BO1(T) cell line was not affected by up to 100 μg/ml PAA in the culture medium, the replication of latent virus DNA appears to be under host cell control rather than virus control.

Arita & Nii (1979) reported that the number of virus antigen-positive producer MSB1 cells is increased by lowering the culture temperature. The same phenomenon has been observed in an Epstein–Barr virus (EBV) producer lymphoblastoid cell line (Hinuma *et al.*, 1967). Lowering the temperature did not increase the amounts of latent MDV genomes in MSB1 cells cultured with PAA or of virus genomes in BO1(T) cells, but increased the rate of MDV DNA replication in MSB1 cells without PAA. These findings also suggest that replication of latent MDV and HVT genomes in BO1(T) cells and of latent MDV genomes in MSB1 cells are controlled by different mechanisms from that of most of the virus DNA in MSB1 cells.

Since the cell population of BO1(T) cells has not been derived from a cloned cell, it is not possible to imply whether both MDV and HVT genomes reside in the same or different cells. Preliminary studies on the subclones of BO1(T) cells indicate that several clones contained both virus genomes. However, some clones of BO1(T) cells contained only MDV genomes. The HVT genomes in these clones could be lost by cloning BO1(T) cells.

The virus rescued from BO1(T) cells by co-cultivation with CEF was HVT, but not MDV (Kitamoto *et al.*, 1980). The failure to rescue MDV from the cells could be due to several factors. Although DNA–DNA reassociation kinetics in this paper suggest that most of the MDV DNA sequences are present in BO1(T) cells, a small portion of the MDV DNA sequences could be deleted in BO1(T) cells and the MDV DNA in the cells could be non-infectious. It is also possible that MDV may be eliminated by passages in CEF cells after co-cultivation of BO1(T) cells with CEF cells.

Silver *et al.* (1979) reported that in an MDV non-productive chicken lymphoblastoid cell line, 12 to 14% of the MDV DNA template was transcribed and only a portion of the virus-specific RNA sequences in whole cells could be detected in the polyribosomal fraction. Similar phenomena have been demonstrated in EBV non-productive human lymphoblastoid cell lines (Hayward & Kieff, 1976). These findings seem to be consistent with ours on the restricted transcription of both the HVT and MDV genomes in BO1(T) cells. However, a portion of the transcripts in BO1(T) cells could be present in very low concentrations and we might have failed to detect them. Possible transcriptional and post-transcriptional control mechanisms require further investigation.

Digestion of BO1(T) cell nuclei with micrococcal nuclease suggested that latent HVT and MDV DNAs possess nucleosomal structures. To exclude the possibility that virus DNA in the nuclei becomes encapsulated and thus protected from digestion, we separated the chromatin subunits from digested nuclei by centrifugation in neutral sucrose gradients.
Nucleocapsids, if present, will sediment to the bottom of centrifuge tubes under these conditions (Fig. 3). In addition, we failed to detect herpesvirus-type particles in BO1(T) cells by electron microscopy (Kitamoto et al., 1980). A nucleosomal structure for latent virus DNA, such as host chromatin, may be necessary for bringing the DNA under host cellular control. Shaw et al. (1979) tested for the presence of nucleosomal EBV DNA in the nuclei of human lymphoblastoid cell lines by a combination of micrococcal nuclease digestion and Southern's transfer and hybridization. They found that the EBV episomal DNA of non-producer Raji cells is folded into nucleosomes, whereas most of the virus DNA of producer P3HR-1 cells and super-infected Raji cells is not. Therefore, the structural organization of latent HVT and MDV DNA is similar to that of latent EBV DNA. In contrast to the state in non-producer cells, a portion of the MDV DNA in the nuclei in producer MSB1 cells was not protected from nuclease digestion. This could be due to a difference in susceptibility to digestion of productive and latent virus DNA in MSB1 cells.

Recently, the intracellular state of MDV DNA in chicken cell lines from MD lymphomas has been studied in two laboratories (Tanaka et al., 1978; Kaschka-Dierich et al., 1979). Tanaka et al. (1978) found that at least 80% of the MDV genomes in a non-producer cell line existed as circular episomal DNA. In contrast, Kaschka-Dierich et al. (1979) reported that a large proportion of the MDV DNA in producer and non-producer cell lines was integrated into cellular DNA and that no circular form of virus DNA was detectable. Conceivably, MDV DNA may be in different forms in different cell lines.

Under present conditions using a small amount of DNA, we showed that at least 20% of the MDV DNA in BO1(T) cells was circular. We could not determine the intracellular state of latent HVT DNA because its content in BO1(T) cells was too low. Since EBV genomes exist as either episomes (Nonoyama & Pagano, 1972; Lindahl et al., 1976) or integrated DNA (Adams et al., 1973), it is possible that the latent HVT and MDV genomes are in similar forms interacting with host cellular chromatin.

The factors influencing restriction of latent HVT and MDV gene expression must be investigated to understand the relation of their expression to virus latency.

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Latency of HVT and MDV genomes


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