Inverted Repeat Regions of Marek's Disease Virus DNA Possess a Structure Similar to That of the *a* Sequence of Herpes Simplex Virus DNA and Contain Host Cell Telomere Sequences

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The genomic structure of Marek's disease virus (MDV) is similar to those of the alphaherpesviruses herpes simplex virus (HSV) types 1 and 2. Sequence analysis of the junction region between the long component (L) and the short component (S) revealed the existence of an *a*-like sequence, similar in structure to the *a* sequence of HSV-1. Further study revealed that the MDV genome contains five copies of the *a*-like sequence within the long terminal repeat region as well as in the short terminal repeat region. The junction between the L and S components was found to contain 10 copies of the *a*-like sequence. Within the *a*-like sequence, a structure homologous to the DR2 of HSV was found to contain 17 copies of the telomeric sequence, GGGGTTA. There appears to be little to no sequence homology between the HSV *a* sequence and the MDV *a*-like sequence; however, the strong physical homology to its counterpart in HSV-1 suggests that the MDV *a*-like sequence may have the same functional homology (the domain for cleavage/packaging of the DNA into the viral capsids and for genomic inversion) as well.

Marek's disease virus (MDV) induces a malignant lymphoma of T cells in chickens (1, 19). MDV has been classified into the *Herpesviridae* subfamily *Gammaherpesvirinae* by its tropism for lymphocytes, the same as for Epstein-Barr virus and herpesvirus of turkeys (HVT) (22). The genome of MDV consists of a linear, double-stranded DNA molecule of approximately 180 kbp (13, 14). The MDV DNA genome is composed of long (L) and short (S) components (3), similar to those of the DNA genomes of herpes simplex virus (HSV) types 1 and 2. Each component consists of a unique region (U_L or U_S) bracketed by terminal (TR_L or TR_S) and internal (IR_L or IR_S) repeats, respectively (3). Specifically, the structure of the HSV DNA genome has been shown to be $ab-U_L-b'a'c'-U_S-ca$ (Fig. 1A) (21, 26). The number of *a* sequences at the junction between the L and S components and at the termini of HSV-1 vary from one to several tandem copies (15, 16, 28). The single *a* sequence of the HSV-1 genome consists of a 20-bp direct repeat (DR1) bracketing a region of 501 bp which contains 19 tandem repeats of a 12-bp sequence (DR2), 3 direct repeats of a 37-bp sequence (DR4), and 2 short stretches of unique sequences (17). The L and S components of the HSV-1 DNA genome have been demonstrated to invert relative to each other, thereby forming four equimolar isomers of the viral genome (10, 21). It has been suggested that the inversion events in the HSV-1 genome result from intramolecular recombination between the termini and the inverted *a* sequences (4). Also, the terminal *a* sequence has been shown to be important for the cleavage/packaging of the viral DNA concatemers (26).

The physical map of MDV has been previously prepared in our laboratory and suggests that the junction region between the L and S components of the viral DNA is contained within the *Bam*HI A fragment (11). We therefore concentrated our studies on the L and S junction and the

terminal repeat regions of the MDV DNA via nucleotide sequencing and Southern blot hybridization.

We now present a detailed report on the existence of an *a*-like sequence which is structurally similar to the *a* sequence found in the HSV genome. This *a*-like sequence is located at the L-S junction and within both terminal repeat regions of the genome.

MATERIALS AND METHODS

Virus propagation and DNA extraction. MDV strain GA clone 21, a pathogenic strain, was propagated in chicken embryo fibroblasts maintained in minimal essential medium with 2% fetal calf serum. When 80% of the cells exhibited a cytopathic effect, virus particles were isolated as previously described (14). Total cellular DNA was extracted with pronase-sodium dodecyl sulfate (SDS) followed by phenol extraction from the MDV GA clone 21-infected chicken embryo fibroblasts, using conditions previously described (14).

Subcloning. By using the cloned *Bam*HI A fragment of MDV (11), the 1.6-kbp *Bam*HI-*Hind*III subfragment of *Bam*HI-A (Fig. 1A) was cloned into both M13 mp8 and mp9 vectors for subsequent nucleotide sequencing.

Nucleotide sequencing. Nucleotide sequences of both strands of the cloned 1.6-kbp subfragment of *Bam*HI-A were obtained by the dideoxy-chain termination method of Sanger et al. (23).

Preparation of probes and Southern blot hybridization. Two subfragments, the 2.3-kbp *Hind*III-*Hind*III and 4.4-kbp *Eco*RI-*Bam*HI subfragments of *Bam*HI-A, were used as probes for Southern blot hybridization. Probes were 3' end labeled by using the Klenow fragment of *Escherichia coli* DNA polymerase I (Bethesda Research Laboratories) and [α - 32 P]dCTP (New England Nuclear). The 30-mer oligonucleotide (GGGGTTA)₅ was synthesized, 5' end labeled with T4 DNA kinase (Bethesda Research Laboratories) and [γ - 32 P]ATP (NEN), and then used as a probe for Southern blot hybridization. Purified MDV viral DNA was cleaved

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with appropriate restriction endonucleases and then separated by electrophoresis through an agarose gel. The DNA was blotted to Nytran membranes (Schleicher & Schuell) as described by Southern (24). When the subfragments of *Bam*HI-A were used as probes, hybridization was carried out at 65°C in the presence of 6× SSC (1× SSC contains 0.15 M NaCl and 0.015 M sodium citrate), 5× Denhardt's solution (1× Denhardt's solution contains 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 0.1% SDS, and 0.05% sodium pyrophosphate. When the synthetic oligonucleotide was used as a probe, the hybridization was performed at 42°C. After hybridization, the membrane was washed twice with 2× SSC–0.1% SDS–0.05% sodium pyrophosphate for 20 min at room temperature and twice with 0.1× SSC–0.1% SDS for 10 min at 52°C or, when the synthetic oligonucleotide was used as a probe, twice with 2× SSC–0.1% SDS–0.05% sodium pyrophosphate for 10 min at 42°C. The membrane was then exposed to X-ray film (Kodak X-Omat) at room temperature.

Nucleotide sequence accession number. The GenBank accession number for the MDV *a*-like sequence is M62517.

RESULTS

Sequence analysis of the 1.6-kbp *Bam*HI-*Hind*III subfragment of MDV *Bam*HI-A. Previous results suggested that the junction region between the L and S components might be present within the left-end portion of the MDV *Bam*HI A fragment (Fig. 1A) (11). We therefore sequenced the 1.6-kbp *Bam*HI-*Hind*III subfragment of *Bam*HI-A. The resulting nucleotide sequence of the *Bam*HI-A fragment is shown in Fig. 1B. Within this region we detected an *a*-like sequence which was structurally similar to the *a* sequence located in the junction region of the L and S components of HSV-1 (17). The *a*-like sequence obtained consisted of 215 nucleotides containing two copies of the 12-bp repeat, GGCCGC GAGAGG, which corresponds to DR1 of the HSV-1 *a* sequence. This sequence also contained a tandem repeat, GGGTTA, reiterated 17 times which corresponds to DR2 of the HSV-1 *a* sequence and two copies of the 13-bp repeat, GGGTTCAGGCCTA, which has been designated as DR4 of the HSV-1 *a* sequence (17) (Fig. 1B). Consistent with the terminology used to describe these regions within HSV, the three different groups of direct repeats in the MDV *a*-like sequence were designated DR1, DR2, and DR4, respectively.

In addition to the repeat sequences, our sequence data revealed the presence of two unique sequences within the region bracketing the 12-bp repeats (DR1) (Fig. 1B). The two unique regions of 7 and 56 bp were followed by the first 13-bp repeat (DR2) or the second, DR1, respectively (Fig. 1B). The

FIG. 1. (A) Genomic and *Bam*HI restriction maps of MDV. The long terminal repeat (TRL), the unique long region 1 (UL1), the direct repeat region (DR), the unique long region 2 (UL2), the long internal repeat (IRL), the short internal repeat (IRS), the short unique region (US), and the short terminal repeat (TRS) of the MDV genome are shown. The a, b, and c represent a, b, and c sequences, respectively. In the *Bam*HI restriction map, the *Bam*HI-A fragment has been enlarged to show the positions of the *Bam*HI, *Hind*III, and *Eco*RI restriction fragments relative to the a, b, c, and US positions. (B) Nucleotide sequence of the region of the *Bam*HI-*Hind*III subfragment of *Bam*HI-A containing the *a*-like sequence. DR1, -2, and -4 are shown. The restriction sites for *Bam*HI, *Hpa*II, *Stu*I, and *Bgl*I are indicated above the sequence. N, unreadable nucleotide.

FIG. 2. (A) Enzyme restriction map of the L-S junction region containing *a*-like sequence concatemers in the MDV genome. The restriction sites for *Bam*HI (Bm) and *Stu*I (S) and sizes of fragments generated with the *Stu*I and *Bam*HI-*Stu*I digestions of genomic DNAs are shown. (B) Southern blot hybridizations using the synthetic oligonucleotide probe (GGGTTA)₅ and the *Stu*I (lane 1)- or *Bam*HI-*Stu*I (lane 2)-digested MDV genomic DNAs. A 1.2% agarose gel was used to electrophoretically separate the restriction endonuclease-digested genomic DNA. Sizes (in base pairs) of resulting fragments are shown.

second unique region (56 bp) was designated U_c according to the HSV-1 *a* nomenclature (18) and contained a stretch of nine thymidine residues (T₉). In the HSV-1 *a* sequence, a CG motif (CGCCGCG) is present in the U_b region (5, 16); in contrast, the MDV *a*-like sequence contained the CG motif (GGCCGCG) within each DR1.

It therefore appears that the 1.6-kbp *Bam*HI-*Hind*III subfragment contains a sequence structurally similar to the HSV-1 *a* sequence.

Determination of the size of the *a*-like sequence in the MDV DNA by Southern blot hybridization. A unit of the *a*-like sequence in the cloned MDV DNA sequence was determined to be 215 nucleotides long. However, the number of GGGTTA sequences within the *a*-like sequence differs between two clones that we have sequenced so far and may represent artifacts of bacterial cloning. We therefore attempted to determine the size of the *a*-like sequence in the genomic DNA of MDV as well as the number of GGGTTA sequences per unit of *a*-like sequence. If the *a*-like sequence is present as concatemers in the genome of MDV as shown in Fig. 2A, cleavage of the genomic DNA with a unique restriction enzyme whose site is located within the *a*-like sequence will generate an *a*-like sequence with a single unit size. Unfortunately, no unique restriction site was found to reside within the *a*-like sequence. However, a *Stu*I restric-

tion site was found in each of the DR4 repeats (Fig. 1B). This restriction endonuclease can generate a fragment of 256 bp containing the regions shown in Fig. 2A. We performed a Southern blot hybridization to determine the size of the *a*-like sequence, using *Stu*I-digested genomic DNA and the synthetic oligonucleotide (GGGTTA)₅ as a probe. The resulting hybridization revealed a strong and broad band of approximately 256 bp and a sharp 4.3-kbp band (Fig. 2B, lane 1). The 256-bp *Stu*I-*Stu*I fragment corresponded to a unit size of the *a*-like sequence containing 26 copies of the GGGTTA sequence. However, since the 13-bp *Stu*I-*Stu*I fragments are not contained within the 256-bp fragment (Fig. 2A), the actual unit size of the *a*-like sequence will be 269 bp (256 + 13). The intensity of the 256-bp band also suggests that MDV contains multiple sets of the *a*-like sequence concatemer or more than two copies of *a*-like sequence in a single concatemer.

To determine whether the 4.3-kbp *Stu*I fragment contains the *a*-like sequence, the genomic DNA was digested with *Bam*HI and *Stu*I. From the nucleotide sequence of the 1.6-kbp *Bam*HI-*Hind*III subfragment (Fig. 1B), double digestion by these enzymes will generate a *Bam*HI-*Stu*I fragment of 357 bp and a *Stu*I-*Stu*I fragment of 256 bp (Fig. 2A). Since both of these fragments are derived from the *a*-like sequence, they should hybridize to the (GGGTTA)₅ probe. As predicted, the (GGGTTA)₅ probe hybridized to the 357-bp *Bam*HI-*Stu*I fragment (Fig. 2B, lane 2) in addition to the 256-bp *Stu*I-*Stu*I fragment. These results indicate that both the 4.3-kbp *Stu*I fragment and the 357-bp *Bam*HI-*Stu*I fragment contain the *a*-like sequence.

The 256-bp *Stu*I-*Stu*I fragment and the 357-bp *Bam*HI-*Stu*I fragment were detected as broad bands by Southern blot hybridization (Fig. 2B). Even when 4% NuSieve GTG agarose (FMC BioProducts, Rockland, Maine) was used for gel electrophoresis prior to Southern blot hybridization, these fragments were detected as broad bands (data not shown). Broadness of these bands might be due to variability of copy number of the DR2, as was observed in the sequence analysis previously described (Fig. 1B).

Localization of the *a*-like sequence in the MDV genomic DNA. If this *a*-like sequence is the counterpart of the HSV-1 *a* sequence, it would also be expected to be present in both terminal regions (TR_L and TR_S). To investigate the localization of the *a*-like sequence, Southern blot hybridizations were performed. Since the complete *Bam*HI restriction map of MDV DNA is known (7), the MDV viral DNA was digested with *Bam*HI and then blotted to a nylon membrane. The autoradiograph obtained upon hybridization of this membrane with the (GGGTTA)₅ probe revealed three bands of approximately 21, 15.5, and 1.52 kbp (Fig. 3, lane 1). Since there are no *Bam*HI restriction sites within the *a*-like sequence (Fig. 1B), this result suggests that the GGGTTA sequence found in the *a*-like sequence localizes to three separate regions within the MDV genome. The size of the largest fragment was approximately 21 kbp, which corresponds to that of the *Bam*HI A fragment. Since our previous report suggested that the 1.6-kbp *Bam*HI-*Hind*III subfragment of *Bam*HI-A contains the L-S junction (11), the region which should contain the *a*-like sequence, hybridization to the *Bam*HI A fragment was expected.

To determine whether either the 15.5-kbp heterogeneous band or the 1.52-kbp band was derived from the TR_S region, we used the 2.3-kbp *Hind*III-*Hind*III subfragment of *Bam*HI-A (a fragment that should be specific for the IR_S and TR_S; Fig. 1A) to probe *Bam*HI-digested genomic DNA. The resulting autoradiograph revealed that the 15.5-kbp hetero-

FIG. 3. Southern blot hybridizations using *Bam*HI-digested viral DNAs. (A) The (GGGTTA)₅ oligomer (lane 1), the 2.3-kbp *Bam*HI-*Hind*III fragment (lane 2), and the 4.4-kbp *Eco*RI-*Bam*HI fragment (lane 3) were used to probe Southern blots of *Bam*HI-digested viral DNA that had been electrophoretically separated in 0.6% agarose gels. Sizes are shown in base pairs. (B) Schematic of the viral genome and locations of the (GGGTTA)₅ probe, the 2.3-kbp *Hind*III-*Hind*III probe, and the 4.4-kbp *Eco*RI-*Bam*HI probe. Locations of the 1.52-, 21-, and 15.5-kbp hybridizing fragments are shown.

geneous band and the 21-kbp *Bam*HI A fragment hybridized with the 2.3-kbp *Hind*III-*Hind*III subfragment. These results suggest that the 15.5-kbp heterogeneous band corresponds to the TR_S region (Fig. 3A, lane 2). When a U_S-specific subfragment of *Bam*HI-A (the 4.4-kbp *Eco*RI-*Bam*HI fragment shown in Fig. 1A) was hybridized to the *Bam*HI-digested genomic DNA, only the 21-kbp *Bam*HI A fragment was observed upon autoradiography. This result confirms that the largest band is *Bam*HI-A and that *Bam*HI-A contains the L-S junction. This result also reconfirms that the 15.5-kbp heterogeneous band covers the TR_S. The absence of the previous *Bam*HI-A subfragment probes to detect the 1.52-kbp band seen in Fig. 3A, lane 1, suggests that like the HSV-1 *a* sequence, the MDV *a*-like sequence must reside within the TR_L region.

Determination of number of *a*-like sequence at the termini and L-S junction region in the MDV genomic DNA. To determine the number of *a*-like sequences within the termini and the L-S junction region, we performed Southern blot hybridization using *Bam*HI-*Bal*I-digested genomic DNA and the (GGGTTA)₅ probe. Since the *Bam*HI restriction site is contained within the *b* and *b'* sequences and the *Bal*I restriction site is contained within the *c* and *c'* sequences

(Fig. 1), digestion of the genomic DNA with *Bam*HI and *Bal*I will generate three bands derived from the TR_L, the L-S junction, and the TR_S regions, respectively. As depicted in Fig. 4B, the TR_L should produce a fragment whose size is 269 times the number of *a*-like sequences plus the distance from the most proximal *a*-like sequence to the *Bam*HI site (173 bp), the L-S junction should produce a fragment whose size is 269 times the number of *a*-like sequences plus the distance from the most proximal *a*-like sequence to the *Bam*HI site (173 bp) plus the distance from the most distal *a*-like sequence to the *Bal*I site (668 bp), and the TR_S should produce a fragment whose size is 269 times the number of *a*-like sequences plus the distance from the most proximal *a*-like sequence to the *Bal*I site (688 bp). Upon digestion of the viral DNA with *Bam*HI and *Bal*I, fragments of 3.53, 2.01, and 1.52 kbp were detected upon hybridization with the (GGGTTA)₅ probe (Fig. 4A, lane 1). The sizes of the *Bam*HI-*Bal*I fragments (1.52 and 2.01 kbp) suggest that the TR_L and TR_S, respectively, each contain 5 copies of the *a*-like sequence, while the 3.53-kbp fragment, derived from the L-S junction, is predicted to contain 10 copies of the *a*-like sequence (Fig. 4A, lane 1; Fig. 4C). Since there is no *Bal*I restriction site within the L terminus of the genome (Fig. 1B), the *Bam*HI-*Bal*I digestion of genomic DNA will generate an L terminus-*Bam*HI fragment. The previous Southern blot hybridization using the *Bam*HI-digested genomic DNA suggested that the 1.52-kbp fragment was derived from the L terminus-*Bam*HI region (Fig. 3). This finding suggests that the 1.52-kbp fragment generated by the *Bam*HI-*Bal*I digestion of genomic DNA corresponds to the L terminus-*Bam*HI fragment. This L terminus-*Bam*HI fragment (1.52 kbp) is 1,345 bp longer than estimated from the nucleotide sequence of the 1.6-kbp *Bam*HI-*Hind*III subfragment of *Bam*HI-A. This 1,345-bp difference in size exactly corresponds to the size of five copies of the *a*-like sequence (269 bp). These results indicate that five copies of the *a*-like sequence are present within the TR_L region.

To confirm whether the TR_L region, the L-S junction, and the TR_S region contain 5, 10, and 5 copies of the *a*-like sequence, respectively. Southern blot hybridizations were performed using *Hpa*II-digested genomic DNA and the (GGGTTA)₅ probe. *Hpa*II restriction sites are located 38 bp upstream and 41 bp downstream from the DR1s in the *a*-like sequence (Fig. 1B). Therefore, *Hpa*II digestion of the viral DNA will generate the following three fragments containing *a*-like sequences: the L terminus-*Hpa*II fragment, the *Hpa*II-*Hpa*II fragment, and the *Hpa*II-S terminus fragment (Fig. 4B). These fragments arise from the TR_L region, the L-S junction, and the TR_S region, respectively. Using the data obtained from the *Bam*HI-*Hind*III subfragment sequence (Fig. 1B and 4B), the size of the *Hpa*II-*Hpa*II fragment from the L-S junction was calculated to be 348 bp if it contained only a single *a*-like sequence. The sizes of the L terminus-*Hpa*II and *Hpa*II-S terminus fragments were calculated to be 307 and 310 bp, respectively, if they also contained only a single *a*-like sequence (Fig. 4B). From the information contained above, if the TR_L region, the L-S junction, and the TR_S region contained 5, 10, and 5 copies of the *a*-like sequence, respectively, then the 307-bp L terminus-*Hpa*II fragment, the 348-bp *Hpa*II-*Hpa*II fragment from the L-S junction, and 310-bp *Hpa*II-S terminus fragment would increase in size to 1,383, 2,769, and 1,386 bp, respectively. When linear viral DNA was digested with *Hpa*II, Southern blotted, and hybridized with the (GGGTTA)₅ probe, two bands of the expected sizes (2.80 and 1.39 kbp) were obtained (Fig. 4A, lane 2). These results suggest that

FIG. 4. (A) Southern blot hybridizations using the (GGGTTA)₅ probe and *Bam*HI-*Bal*I (lane 1)- or *Hpa*II (lane 2)-digested MDV genomic DNA. A 1.2% agarose was used for gel electrophoresis to separate enzyme-digested genomic DNA. The viral DNA in lane 1 was digested with *Bam*HI-*Bal*I, the viral DNA in lane 2 was digested with *Hpa*II, and the MDV-infected cell DNA in lane 3 was digested with *Bal*I. (B) Structure of a single *a*-like sequence and locations of the *Bam*HI and *Bal*I restriction sites which are located to the regions flanking the *a*-like sequence. (C) Schematic interpretation of the results shown in panel A. The large boxes represent the TR_L, IR_L, IR_S, and TR_S as depicted in Fig. 1A. The small boxes labeled 1 to 10 represent a single *a*-like sequence contained within each of the regions.

MDV contains 5 copies of the *a*-like sequence within the TR_L and TR_S regions and contains 10 copies of the *a*-like sequence within the L-S junction.

To further confirm the number of *a*-like sequences within the TR_L and TR_S regions, MDV-infected cell DNA containing MDV in the circular form was digested with *Bal*I, blotted to a nylon membrane, and hybridized with the (GGGTTA)₅ probe. A single band of 3.50 kbp was detected (Fig. 4C). No band of this size was detected when chicken embryo fibroblast DNA was hybridized with the same probe (data not shown). The presence of this single 3.50-kbp band suggests that when the TR_L and TR_S regions are joined to produce the circular form of MDV, a single *Bal*I-*Bal*I fragment (the same size as the *Bal*I-*Bal*I fragment generated within the L-S region) is produced. The two resulting fragments are therefore visualized as a single band upon hybridization. The size of the fragment generated from the TR_L-TR_S joining region shows that it contains 10 copies of the *a*-like sequence (5 copies from the TR_L region and 5 copies from the TR_S region). We therefore concluded that the previously unknown location of the *Bal*I restriction site, lying outside the *a*-like sequence, is contained within the adjoining *b* and *b'* regions and that the TR_L and TR_S regions each contain five copies of the *a*-like sequence.

DISCUSSION

We have demonstrated that MDV possesses an *a*-like sequence composed of 2 copies of DR1, 17 to 26 copies of DR2, 2 copies of DR4, and two unique regions (U_b and U_c). This sequence is structurally similar to that of the HSV-1 *a* sequence (17). The S terminus of the HSV-1 genome contains only one copy of the *a* sequence (16, 28), while our results show that five copies of the *a*-like sequence are

present in the S terminus of the MDV genome. However, in the Southern blot hybridization using the (GGGTTA)₅ probe, *Bam*HI-digested viral DNA generated a broad 15.5-kbp band (Fig. 3, lane 1) which was derived from the S-terminus region. This broad band might reflect the variability of copy number of the DR2 within the *a*-like sequence or a variable number of *a*-like sequences within the S terminus. We also found the L terminus and the L-S junction region to contain 5 and 10 copies of the *a*-like sequence, respectively. Previous reports (16, 17, 28) have shown that the copy number of *a* sequences within HSV-1 varies considerably. In contrast, our study suggests that the copy number of *a*-like sequences within the majority of MDV species is relatively stable.

Within the second unique region (U_c) of the *a*-like sequence (Fig. 1B), a T₉ sequence was located 11 bp upstream of the second DR1. Similarly, T₆ sequence is located 14 bp upstream of the second DR1 in the HSV-1 *a* sequence (17). Deiss et al. (5) have demonstrated that several stretches of thymidine residues (T_n) are conserved in the following herpesviruses: HSV-1, Epstein-Barr virus, bovine herpesvirus, varicella-zoster virus, and herpesvirus saimiri. It has also been demonstrated that the conserved T_n sequences are located 30 to 35 nucleotides upstream of the viral DNA cleavage sites in all of the aforementioned herpesviruses. In addition to the T₉ sequence, the MDV *a*-like sequence contains a C+G-rich sequence (GGCCGCG) in each DR1. The CG motif of HSV-1 (CGCCGCG) is conserved and is close to the cleavage sites in the genomes of several of the herpesviruses mentioned above (5). These findings suggest that the *a*-like sequence may function as a cleavage domain for the MDV viral DNA as do the *a* sequences of other herpesviruses.

As with the HSV-1 DR2 sequence, the number of DR2s within the MDV *a*-like sequence was found to be variable: 19 to 22 copies within HSV-1 (17) and 17 to 26 copies within MDV. The GGGTTA repeat found within the DR2 of the MDV *a*-like sequence is analogous to the DR2 found in the HSV-1 *a* sequence (17). However, the nucleotide sequences of the DR2s within MDV and HSV-1 are different. In contrast, we have found that the tandem GGGTTA repeat sequence has been conserved within human herpesvirus 6 (HHV-6) and HVT (11). The (GGGTTA)₅ probe hybridizes to the HVT *Bam*HI F fragment (unpublished data), a fragment suspected to be located within the L-S junction region of HVT (9). The exact location of the GGGTTA sequence within the HHV-6 viral genome remains to be determined. Surprisingly, the HVT *Bam*HI F fragment contains the GGGTTA sequence repeated 120 times (unpublished data). From these findings, it is possible that the conserved GGGTTA tandem repeats within the MDV, HHV-6, and HVT genomes may function as an important factor for the inversion events ascribed to the DR2 region within the HSV-1 *a* sequence (4).

Several studies of the *a* sequence within HSV-1 have been reported by many investigators (4, 5, 10, 16, 17, 21, 26–28). Most of them have suggested that the *a* sequence is involved in the cleavage/packaging process during viral replication and in the inversion events seen within the HSV-1 genome. The inversion events result in the formation of four equimolar isomers of the HSV-1 viral DNA (6, 8). In this study, we attempted to show that inversion events also occur within the MDV genome. Unfortunately, in contrast to HSV-1, the complete sequence of the MDV viral genome is not known, and we were unable to locate restriction endonuclease sites that would allow us to determine whether inversion occurred. However, we have now shown that MDV contains an *a*-like sequence which is structurally homologous to the HSV-1 *a* sequence.

Interestingly, the GGGTTA sequences within the MDV DR2 region and within HVT and HHV-6 have been found to be highly conserved in the telomeric sequences of human chromosomes (18, 20) as well as in the telomeric sequences of lower eukaryotes (18). The recently published DNA sequence of a human telomeric region (2) reveals the presence of a structure remarkably similar to the herpesvirus *a* sequence. This human telomeric sequence contains several blocks of repeated sequence separated by regions of unique DNA analogous to the herpesvirus U_b and U_c. There even exists a block of five 37-bp repeats in a position analogous to the three 37-bp DR4 repeat sequences. The repeat regions in both the herpesviruses and the human telomere consist of predominantly G+C-rich sequences. Since one of the properties of the telomere is to ensure complete replication of the chromosomal DNA, it is interesting to find this telomeric sequence within the region of MDV thought to be critical for the processing of replicated viral DNA. The presence of this telomeric sequence in a virus that is maintained as an episome in immortalized lymphocytes, is apparently randomly associated with host chromosomes, and whose replication is tightly regulated by the cell (12, 25) suggests that the cell may recognize MDV as a minichromosome and treat it as such. Since there exists a telomerase within the cell that is responsible for synthesis of the GGGTTA sequence, it will be interesting to determine what if any role the cellular telomerase plays in the replication of MDV. Sequencing of the chicken telomeric and telomere-associated DNA may provide some clues as to the origin of this sequence within MDV.

Further work will be required to determine whether the telomere-like sequence has any function in the life cycle of MDV and whether the *a*-like sequence is functionally homologous to the *a* sequence present within HSV-1.

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