THE DYNAMIC HERPESVIRUS DNA GENOME: THE CASE OF MDV-1 AND HSV-1

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Summary. – Herpesviruses evolved from an ancestral viral genome that contained five blocks of genes which provide the members of this family of viruses with structural and enzymatic properties. These genes allow the herpesviruses to infect a host by entering into the nuclei of the cells, the site of replication and transcription of the viral DNA. The viral mRNAs are released into the cell cytoplasm where synthesis of enzymatic and structural proteins occurs. The latter proteins are responsible for the formation of the infectious virions. Herpesviruses that were able to adapt to different hosts during the evolution of the species (speciation) had acquired additional genes from transposons or retrotransposons that allowed them to successfully maintain their hold in the specific vertebrate host. The present overview deals with molecular differences between Marek's disease virus type 1 (MDV-1) and herpes simplex virus type 1 (HSV-1) and the specialized genes that differentiate MDV-1 from HSV-1, the promoters of the viral genes that control gene expression and the nuclear localization signals. Dynamic changes in the viral genomes that may occur during viral DNA replication and recombination and their effects on virus pathogenicity and genome evolution will be discussed.

Key words: MDV-1; HSV-1; DNA genome; evolution of herpesviruses; DNA replication; DNA recombination

Introduction

The advances in the sequence analyses of the DNA genomes of members of the *Herpesviridae* family of viruses provide new information on the nonstructural and structural viral genes and the functional genomic domains. These studies, as well as the analyses of the viral proteins and their functions during the virus growth cycle in cultured cells *in vitro*, and in experimental animals *in vivo*, added much to the understanding of the molecular biology of this family of viruses that include the pathogenic and apathogenic MDV-1 and HSV-1, the subjects of the present overview.

Three topics related to MDV-1 and HSV-1 pathogenic and apathogenic viruses will be discussed:

A) Evolutionary trends of the viral DNA genomes: Although the general organization of the two viral DNA genomes contains unique long (U_L) and unique small (U_S) sequences with the „a" sequence between the internal repeat of U_L (IR_L) and the internal repeat of U_S (IR_S), they differ in having the origins of replication (ORI_L) in MDV-1 located in the IR_L and TR_L repeats of U_L while in HSV-1 genome, two origins of replication (ORI_S) are positioned in the terminal repeats (IR_S and TR_S) of U_S sequence and a third origin of replication (ORI_L) is located in the middle of U_L. Contrary to HSV-1, MDV-1 contains the telomeric sequence $(GGGTTA)_{26}$, which was also found in the genome of human herpes virus-6 (HHV6). The evolution of MDV-1 genome and genes and their relatedness to chicken DNA with immediate early (IE) genes promoters with attachment domains for the chicken transcription factors and the evolution of HSV-1 genes and promoters recognized by mammalian transcription factors will be discussed.

B) MDV-1 and HSV-1 pathogenicity genes: MDV-1 and HSV-1 markedly differ in the mode of infection of their hosts, the choice of target cells and in the effects on the host immune systems. MDV-1 infection leads to latency

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#Invited lecture dedicated to the memory of M. Nonoyama.

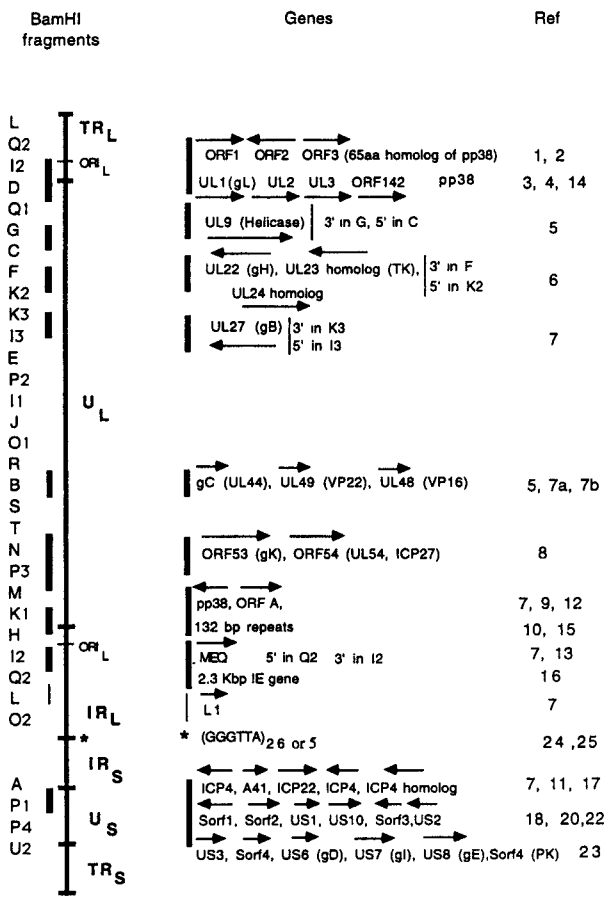

Fig. 1
The MDV-1 genome and genes (1998)
The virus genes that were characterized by sequencing of *Bam*HI restriction DNA fragments was mapped along the MDV-1 genome. Arrows indicate the direction of gene transcription. The numbers of the references are as follows: 1) Bradley *et al.*, 1989; 2) Becker *et al.*, 1994; 3) Yoshido *et al.*, 1994; 4) Makimura *et al.*, 1994; 5) Wu *et al.*, 1996; 6) Shimojima *et al.*, 1997; 7) Omar and Schat, 1996; 7a) Koptidesová *et al.*, 1995, 8) Ren *et al.*, 1994; 9) Cui *et al.*, 1991; 10) Iwata *et al.*, 1992; 11) Cantello *et al.*, 1997; 12) Ono *et al.*, 1994;13) Jones *et al.*, 1992; 14) Zhu *et al.*, 1994; 15) Chen and Velicer 1991; 16) Hong and Coussens, 1994); 17) Cantello *et al.*, 1994 18) Parcell *et al.*, 1994; 19) Urakawa *et al.*, 1994; 20) Jang *et al.*, 1997; 22) Brunovskis and Velicer, 1995; 23) Brunovskis and Kung, 1996; 24) Kishi *et al.*, 1988; 25) Kishi *et al.*, 1991.

in and to transformation of T cells in addition to other types of pathology while HSV-1 is capable of latent infection in the nervous system. The role of the dendritic cells system in the induction of the immune protection against MDV-1 and HSV-1 in chickens and in humans will be analyzed.

C) Modifications in the MDV-1 and HSV-1 viral genomes leading to attenuation: amplification of the 132 base pair repeat sequences near the TR_L ORI_L of the very virulent MDV-1 genome after serial passages in cultured chick embryo fibroblast (CEF), resulting in attenuated virus and a similar amplifications in the naturally attenuated apathogenic vaccine virus isolate (CVI 988, Rispens), led to experiments to attenuate vvMDV-1 by serial passaging of the pathogenic virus in CEF *in vitro*. Although apathogenic HSV-1 isolates were reported, an attenuated HSV-1 virus for use as a human vaccine is not yet available. Studies on an HSV-1 recombinant that lacks pathogenicity to mice revealed rearrangements in the viral DNA genome. The molecular basis for the plasticity of the HSV-1 DNA genome leading to intramolecular recombination and to the elimination of pathogenicity genes will be presented.

I. The MDV-1 DNA genome and its known genes

MDV-1 genome and the *Bam*HI fragments are presented in Fig. 1 which provides the organization of the viral genes that have been reported. With the exception of ORF1 (Becker *et al.*, 1992, 1993), MDV-1 genes were reported to be homologous to similar genes in HSV-1 DNA genome. Although the two viral DNA genomes resemble each other in having the U_L and the U_S sequences that are flanked by repeat sequences with an „a" sequence between the IR_L and IR_S, there are specific features in the MDV-1 genome that differentiate it from the HSV-1 genome:

a) The origins of DNA (ORI_L) replication, are situated in MDV-1 terminal and internal repeats of the long unique sequence (TR_L and IR_L) while in HSV-1 DNA there are three origins of DNA replication: ORI_L in the middle of the U_L and two ORI_S that are positioned in the repeat sequences (IR_S and TR_S) of the unique short (U_S) sequence. The nucleotide sequence of MDV-1 ORI_L and HSV-1 ORI_S are identical (Bradley *et al.*, 1989);

b) The MDV-1 IR_L contains a telomeric repeat sequence $(GGTTA)_n$ (n = 26 and 5 in two different virus isolates), similar telomeric sequences were reported in the genome of HHV6. In contrast, HSV-1 DNA contains short monomeric nucleotide sequences that may be incomplete or rudimentary telomeric sequences;

c) MDV-1 DNA contains tandem repeats of 132 bp that are positioned in the U_L DNA sequence near the ORI_L in the IR_L. Such a tandem repeat is not present in HSV-1;

d) MDV-1 genome contains the MEQ gene (Omar and Schat, 1996; Jones *et al.*, 1992) that is involved in the tumorigenic transformation of T cells in infected chickens while HSV-1 does not have a defined oncogene. It is logical that both MDV-1 and HSV-1 will have similar genes that encode enzymes, nonstructural and structural proteins. The differences between the MDV-1 and HSV-1 homologous genes may reside in the control of gene expression and in the antigenicity of the homologous viral proteins.

Table 1. Comparison between transcription factors binding domains in promoters of MDV-1, MDV-3 and HSV-1 genes

A.								
HSV-1, U_L54 promoter: transcription factors binding domains								
Oct 4, Oct 1-IE2, Sp1-IE3, Sp1-CS1, Sp1CS2, Y box								
MDV-1, U_L54 homolog								
OctA1.1, OCTA3, NF-E1_CS1, MAT-OCTA2, NF-A1-IgHC								
IgHC.14; 15; 16, U5snRNA, Ig Kappa2, Oct R_CS/Rev,								
keratinocytes enhancer, OctA_(1)								
B.								
MDV-2, U_L27, gB homolog								
beta-globin_US, AP4, CRE 2, E4TF1_CS, NF-D1.5, CREB_CS1								
c-fos_US/Rev, IE1.2, c-mos_DS1/Rev, SV40.6/Rev, malT_CS								
E4F1_CS, E4F1-E4.4, HSV_IE repeat, AP-3_CS/Rev								
MDV-3, U_L27(gB) herpesvirus of turkeys								
GCRE, beta-globin_US, GCN4-HIS, malT_CS, AP-1_CS3, NF-E1_CS2/Rev								
E1A-F_CS, E2A_CS, F-ACT-1±RS, Sp1-SiteC/Rev								
C. MDV-1 ORF-1								
TATA-box.1, NF-E1_CS1, GAL1-TATA, hsp70.5, c-mos-DS1, malT-CS/Rev,								
NF-E1_CS2/Rev, PEA3_CS, ICP4_IE3, EcR-consensus, SEF4-consensus								
D. HSV-1 U_S12 (88 aa)								
101 domains in the promoter for binding of transcription factors								
E. Human insulin gene promoter in pancreatic β cells								
Factors:	Sp1, IUF1	OCT-1	USF	IUF1	CREB	IUF1	IEF1	Pur-1
Sites:	Sp1, A5	NRE	E2	A3	CRE/CCAAT	A2	E1	G1,TATAA

Table 2. Comparison of nuclear and nucleolar localization signals in the U_L54 gene of HSV-1 and MDV-1

A. Nuclear localization signal
HSV-1 U_L54 (141) RRGRRRGRGRGG (152)
MDV-1 U_L54 (174) RRLQEGHHRRRFYSERR (190)
B. Nucleolar localization signal
HSV-1 U_L54 (111) RRPSCSPEQ (119)
MDV-1 U_L54 (138) RRNFPMSPTSQ (148)

One of the differences between the two viruses may reside in the promoters of the nonstructural and structural viral genes and in the domains for the binding of nuclear transcription factors. Table 1A shows that the promoter of the MDV-1 U_L54 (immediate early (IE-2)) gene and HSV-1 U_L54 gene differ in their transcription factors binding domains. It may be of interest that the promoter of MDV-1 U_L54 gene contains a keratinocytes enhancer sequence that may explain the ability of MDV-1 to have a lytic replicative cycle in the feather follicles. A difference between the transcription factor domains in the promoter of U_L27 gene (the gB gene) of MDV-1 and MDV-3 is shown in Table 1B. The promoter of MDV-1 ORF-1 (Becker *et al.*, 1994) shares a transcription factor domain with MDV-2 U_L27 gene promoter. The selection of promoter sequences during species evolution that will determine the organ-specific expression of genes can be seen in Table 1E, revealing specific sequence domains for binding transcription factors in the promoter of the human insulin gene that restricts the production of this hormone to β cells of the pancreas only (Doherty, 1997). The promoter of HSV-1 U_S12 gene, which codes for an 88 amino acid peptide that blocks in the infected cells the transporter proteins TAP1 and TAP2 from transporting the nonapeptides from the cytosol to MHC class I molecules in the endoplasmic reticulum (ER) of HSV-1 infected cells, was found to have 101 putative transcription factor binding domains. The large number of binding domains may ensure the expression of the HSV-1 U_S12 gene in different cell types in the infected host. MDV-1 is not known to have a homologue of the HSV-1 U_S12 gene.

A difference was noted in the nucleotide sequences coding for the nuclear and nucleolar localization amino acid signals in the ICP27 protein coded by U_L54 genes of HSV-1 and MDV-1 (Table 2). This difference may indicate that further exploration of the differences between the promoters of MDV-1 and HSV-1 homologous genes and the functional signals in their encoded proteins may provide a better understanding of the differences in the host-dependent regulation of the herpes viruses that infect different hosts such as chickens and humans.

II. Evolution of herpesviruses and the possible origins of MDV-1 and HSV-1 DNA genomes

The molecular evolution of herpesviruses was studied by Karlin *et al.* (1994) who based their analysis on genomic and protein sequence comparisons. The authors proposed

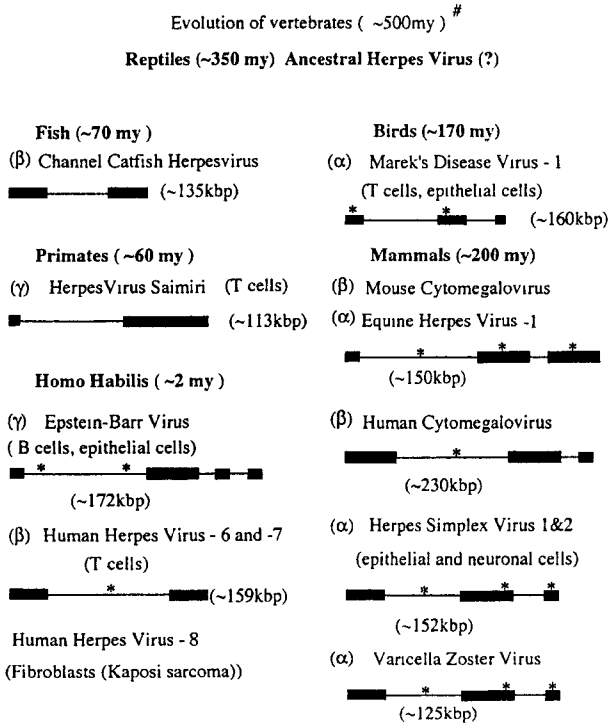

Fig. 2
Steps in the evolution of herpesviruses
The evolution of vertebrates that began around 500 my ago is believed to coincide with the beginning of the evolution of herpesviruses from an ancestral virus, (possibly a reptile herpesvirus). The herpesviruses lacking the U_S sequence are shown as one branch and herpesviruses that had acquired the U_S genes start with MDV-1. The asterix (*) denotes the position of ORIs in the viral genomes. The periods of mass extinction that led to disappearance of forms of life occurred 438, 367, 248, 208 and 65 my ago.

Table 3. Blocks of genes in the U_L sequence shared by all herpesviruses

Block	α, β, γ genes in the U_L sequence
I	42 β, 39 α,β, 38 γg2, 37 γ1, 36 γ2, 35 γ2, 34, 33, 32 γ2, 31 γ2
II	30 β, 27 β, 28 β, 29 β
III	54 α, 52 β, 51 γ1, 50 β, 49 α,β
IV	22 γ2, 24 b,γ1,γ2, 25 γ1, 26 γ2
V	18 γ1, 19 γ1
VI	15 Ex2 γ1, 17 γ1, 16 γ1, 15 Ex1 γ1, 14 γ1, 13 γ1, 12 β, 10 γ2, 9 β 7 γ1, 6 γ1, 5 β
VII	2 β, 1 γ1, γ2

By Gompels *et al.*, Virology **209**, 29–51, 1995.

that the U_S segment of herpesviruses evolved from a transposon or a retrotransposon that was attached or inserted into an ancestral herpesvirus genome by a nonhomologous recombination event at or near the terminal repeats following circularization of the genome in conjunction with viral DNA replication. Karlin *et al.* (1994) placed MDV-1 closest to HHV6 rather than to any alpha herpesvirus. On the basis of protein similarities, the authors concluded that the ancestral herpesvirus gave rise to HHV6 that further evolved to form the human cytomegalovirus (HCMV). The alpha herpesviruses were hypothesized to be of more recent ancestry and equine herpes virus-1 (EHV1) stood out as a most central alpha herpesvirus with stronger similarities in its DNA and proteins to other alpha herpesviruses. Herpesvirus saimiri and Epstein-Barr virus (EBV) were though to constitute a separate branch in the evolution of herpesviruses.

A hypothesis that the ancestral herpesvirus genome yielded two branches of evolution of herpesviruses is described in Fig. 2. The herpesviruses that contain only a unique (U_L) DNA flanked by two inverted repeat sequences were arranged according to the evolutionary time scale of their hosts. The herpesviruses that have both U_L and U_S flanked by their repeat sequences were also arranged according to the timetable of the evolution of their hosts. It is suggested that the ancestral herpesvirus genome acquired the U_S DNA and the genes encoded by them before or during the adaptation of MDV-1 to birds that evolved 170 million years (my) ago. The channel catfish herpesvirus of fish that evolved 70 my ago was found to lack the U_S sequence. The herpesviruses that have the U_L DNA infect and transform T or B cells (herpesvirus saimiri, human EBV, respectively), infect T cells (HHV6, HHV7) or fibroblasts (HHV8). In contrast, the herpesviruses that contain U_L and U_S DNA genes are capable of infecting and completing their life cycle in many cell types in the infected host. MDV-1 infection of T cells leads to transformation of these cells without virus replication. Virus replication occurs in the chicken skin feather follicle cells, the site of virus maturation. Similarly, HSV-1 abortively infects T cells *in vitro* but infects and produces virus progeny in many cell types in the infected host.

It is not yet possible to conclude when the ancestral herpesviruses had evolved but it may be possible to assume that the herpesviruses existed in vertebrates that had started to evolve at about 500 my ago. It is possible to assume that the two types of the herpesvirus U_L and U_L+ U_S genomes continued to evolve during the evolution of the vertebrate species (speciation). When the organization of DNA genomes of herpesviruses that were isolated from lower life forms (e.g. clams) and from reptiles will be deciphered, it may be possible to better define the evolution of the herpesvirus family.

The sequencing of HHV6 genome and the comparison of the genes in the U_L DNA to the genes in the U_L DNA of beta, gamma and alpha herpesviruses enabled Gompels *et al.* (1995) to determine which of the viral genes contribute to the common virus structural organization and which genes were acquired during evolution of the viruses in the course speciation. The HHV6 U_L genes were compared by Gompels *et al.*

Table 4. Acquired genes in HSV-1 genome not present in the HHV6 DNA genome

A. Genes incorporated into the U_L DNA	
1.	U_L8 β, U_L11 γ1, U_L3 γ2, U_L4 γ1
2.	U_L20 γ1, U_L21 γ1 , U_L23 β (TK)
3.	U_L40 β, U_L41 γ1, U_L43 β, U_L44 γ2 (gC), U_L45 γ2, U_L46 γ1, U_L47 γ2, U_L48 γ1
4.	U_L53 γ2 (gK), U_L55, U_L56
B. Genes incorporated into the U_S DNA	
1.	U_S1 α, U_S2 γ1, U_S3 β, γ1
2.	U_S4 γ1 (gG), U_S5 (gJ), U_S6 β, γ1 (gD), U_S7 γ1 (gI), U_S8 γ1 (gE), $U_S8.5$ γ1, US9 γ1
3.	U_S10 γ1, U_S11 γ2
5.	U_S12 α

By Gompels *et al.* (1995) and Ward and Roizman (1995).

(1995) to the U_L genes of HCMV, herpesvirus saimiri (HVS), EBV, EHV1, varicella-zoster virus (VZV) and HSV-1. The authors identified seven blocks of genes in the genomes of all these herpesviruses, as shown in Table 3. It was possible to conclude that 2 alpha genes, 14 beta genes and 22 gamma genes, altogether 38 common genes, are shared by all the herpesviruses that were studied. In the DNA genome of each virus prototype the seven blocks of genes were found to be differently arranged.

Unfortunately, the complete sequence of MDV-1 is not yet available and therefore it is not yet possible to define the genes that this virus acquired during the adaptation of its ancestral herpesvirus to chickens. Comparison of HHV6 genes to HSV-1 genes aids in determining which of the genes were acquired by the HSV-1 DNA genome during its evolution. Table 4 lists the acquired HSV-1 genes that were incorporated into the U_L DNA and the genes in the U_S DNA sequence of the viral DNA genome, respectively. It can be noted that three beta genes that code for subunits of viral enzymes (U_L8, U_L40, and U_L23) were added to the HSV-1 genome. One beta gene (U_L43) encoding a protein that has at least six transmembrane domains, and twelve gamma 1 and gamma 2 genes were added to the U_L genome, including the two glycoprotein genes for gC (U_L44) and gK (U_L53) as well as additional gamma genes that code for structural proteins. The genes in the HSV-1 U_S DNA, designated block VIII in Table 4B contain five glycoprotein genes: U_S4 (gG), U_S5 (gJ), U_S6 (gD), U_S7 (gI) and U_S8 (gE), and three tegument proteins (U_S9, U_S10 and U_S11). The U_S genes that encode the glycoproteins are also present in the U_S DNA sequence of MDV-1 indicating that the U_S DNA sequence was introduced in the herpesvirus DNA during or before the evolution of birds.

A unique gene that was acquired by the HSV-1 U_S DNA (block VIII) is the U_S12 gene, an alpha gene that encodes an 88 amino acid protein, which enables HSV-1 to partly prevent of the induction of cytotoxic T cells (CTLs) in the infected host (Hill *et al.*, 1995).

Another gene that HSV-1 may have acquired from the infected cellular genome is the gamma 1 gene 34.5 (McGeoch and Barnet, 1991) whose 63 amino acid residues of the carboxy terminus are 83% identical to a similar amino acid sequence of the MyD116 cellular gene in the mouse genome. The 34.5 gene was reported to trigger total shutoff of protein synthesis in HSV-1-infected neuroblastoma cells (Chou and Roizman, 1992).

III. The life cycle of MDV-1 in infected chickens and the development of attenuated virus strains

The life cycle of MDV-1 in infected chickens was described by Payne (1985). The infection of chickens starts with the entry of the MDV-1 into the respiratory tract of the chicken followed by virus infection of the lymphoid tissue and bone marrow causing a functional loss of B and T cells, an early immunosuppression, cell-associated viremia and T-lymphocytosis leading to leukemia. The viremia allows the virus to reach the feather follicles, the site of virus maturation. In recent years, the research on the dendritic cells in vertebrates and humans had led to the understanding of the functions of these cells that are made in the bone marrow, released to the blood stream and mature in all the epithelia of the vertebrate. The mechanism of the presentation of antigens by dendritic cells and their ability to induce the two arms, the humoral and the cellular, of the immune response in vertebrates was reviewed by Banchereau and Steinman (1998). Little is known about the dendritic cells in chickens (Davidson *et al.*, 1994), and their role in the induction of the immune responses in the MDV-1-infected chickens. The dendritic cells may be involved in the induction of antiviral CTLs in Marek's disease and in chickens vaccinated with a nononcogenic MDV vaccine (Schat, 1991; Morimura *et al.*, 1996; Omar and Schat, 1997). There is a need for studies on the chicken dendritic cell system to understand its role in vaccine-induced protection of chickens against infection by MDV-1.

Anti-MDV-1 vaccines to protect chickens against disease were developed shortly after the discovery of MDV-1 to reduce the economic losses to farmers. These vaccines included the herpesvirus of turkeys and attenuated MDV-1 isolates (Witter, 1985). The study of CVI988 Rispens vaccine, an apathogenic natural isolate of MDV-1 revealed that attenuation of this virus may be connected with amplification of the 132 bp repeats in the MDV-1 genome (Fig. 1) due to a marked change in the transcription from the virus gene that contains the 132 bp tandem repeats as an intron (Bradley *et al.*, 1989). Subsequent passaging of a very vir-

ulent MDV-1 in CEF *in vitro* led to the amplification of 132 bp tandem repeats in the viral genome (Becker *et al.*, 1993). It was suggested that the amplification of the 132 bp tandem repeats might result from a change in the DNA replication at or near the origin of replication (ORI_L) in the IR_L of MDV-1 DNA (Becker *et al.*, 1993). With the help of the polymerase chain reaction (PCR), it was possible to differentiate between pathogenic serotype MDV-1 and vaccine viruses of MDV serotypes 2 and 3 (Becker *et al.*, 1992).

It is possible that the MEQ gene of MDV-1 that encodes a 339 amino acid protein with N-terminal basic leucine zipper (bZIP) had been acquired from a host cell genome since the MEQ gene was reported to be homologous to other bZIP proteins, members of the Jun/Fos family of transcription factors (Liu *et al.*, 1997). The insertion of reticuloendotheliosis virus long terminal repeats (LTRs) into the MDV-1 genome was reported by Isfort *et al.* (1994) and Brunovskis and Kung (1996) to occur in the IR_S and TR_S sequences. These studies may be taken to signify the importance of recombination events between nuclear retroelements, retrons or retroviruses present in the chicken cell nuclei in the shaping of the herpesvirus genome of MDV-1.

IV. The life cycle of HSV-1

HSV-1 is capable of infecting mucosal, epithelial and neuronal cells in its human host usually with a lytic infection in the cells, except the neurons in which a latent virus infection is evident. Infection of neurons in trigeminal ganglia leads to latency of HSV-1 that can be reactivated by external as well as internal induction and triggering mechanisms that are not yet understood. In spite of the fact that humans are sensitive to infection with HSV-1 during their life, a protective anti-HSV-1 vaccine for the immunization of children and adults against HSV-1 infection is not available. A small percentage of infected humans develop a latent infection in the brain amygdala and hippocampus (Becker, 1995) and most infected people suffer from reactivation of the latent virus.

Herpesviruses had acquired the nucleotide sequences that allow the viral genome to perform intramolecular recombination of the DNA termini with nucleotide sequences located between the internal repeats (IR_L and IR_S) of the U_L and the U_S segments of the DNA genome. These recombination events result in the formation of circular-linear DNA molecules (Umene, 1998). The description of HSV-1 circular-linear DNA molecules that were isolated from infected nuclei and viewed by electron microscopy (Becker, 1978) indicates that intramolecular recombination is a common event during HSV-1 DNA replication. During the replication and recombination of HSV-1 DNA there may be opportunities for nuclear retroelements or retrons that may carry cellular genes to recombine with the viral DNA. Such recombination events may lead to the insertion of cellular genes, or portions of cellular genes, near or inside of viral genes. Herpesvirus genomes which acquired cellular genes that provide the herpesvirus with a better capability of interfering with the host immune system (e.g. HSV-1 U_S12 gene) may have a selective advantage in the adaptation to their host.

It is possible that the intramolecular recombination events in the replication of HSV-1 DNA molecules result in deletions, insertions or amplifications of parts of the virus genome. Such events may lead to the generation of apathogenic HSV-1 virus that may be useful for the development of a human anti-HSV-1 vaccine, as well as to more pathogenic viruses. The evolution of the herpesvirus genome is still continuing.

V. HSV-1 DNA replication and intramolecular recombination

Studies on the replication of HSV-1 DNA in infected cells *in vitro* were recently reported by Severini *et al.* (1996) indicating that „more than a decade ago it was proposed (Becker *et al.*, 1978; Jacob *et al.*, 1979) that HSV replicates through a rolling-circle mechanism, i.e. DNA synthesis begins at a nick at the origin of replication (ORI) and the replication fork moves endlessly around a circular genome to produce head to tail repeats (concatamer) of the monomer genome". Severini *et al.* (1996) quoted our studies where we defined the rolling-circle mechanism of DNA replication as the mechanism of synthesis of defective HSV-1 DNA (Becker *et al.*, 1978). As a result of the formation of circular DNA molecules that include an ORI_S sequence, tandem repeats of the small HSV-1 DNA sequence derived from the viral genome near an ORI_S sequence are amplified to yield long defective DNA molecules that are cleaved to the size of the normal HSV-1 DNA genome (Becker *et al.*, 1969) and packaged into the viral capsids and virions.

The mechanism of the HSV-1 DNA genome replication was reported by Shlomai *et al.* (1976) and in a further study by Friedman *et al.* (1977). Analysis of replicating HSV-1 DNA molecules that were viewed by electron microscopy led to the identification of „four classes of virus DNA molecules: (a) mature linear DNA molecules, 52.4±3.3 mm in length, (b) DNA molecules that contain a replicative loop or are Y-shaped, resembling replicative intermediates; (c) virus DNA molecules having one or more single-stranded filaments attached to them, and (d) molecules with collapsed regions or with branches. A few circular molecules as well as linear DNA molecules longer than unit length were observed. The virus DNA molecules resembling replicative intermediates gradually increased in number and reached a maximal amount of 5% of the virus DNA population at 12 hrs post infection.

Table 5. Amplification of MDV-1 and HSV-1 genomic sequences near the origin of DNA replication

1. MDV-1
a. Amplification of 132 bp sequence near the ORI_L in the ITR_L
2. HSV-1
a. Amplification of U_S sequence near the ORI_S1 in the ITR_S and further amplification of the vDNA by the rolling circle mechanism generating defective HSV-1 genomic DNA
b. HSV-1 (HFEM) – Amplification of the ORI_S sequence (472 bp)
c. HSV-1 (R15 recombinant) – Amplification of the U_S DNA containing U_S1 and U_S2 and insertion into the deletion in the DNA near ORI_S in the TR_S

It was therefore of interest that twenty years after the publication by Shlomai *et al.* (1976). Severini *et al.* (1996) reported that replicating HSV-1 DNA molecules with „branched" structures were also visualized by electron microscopy. Molecules with a single Y junction were observed as well as large tangles containing two or more consecutive Y junctions. It was concluded by Severini *et al.* (1996) that their findings „add complexity to the simple model of rolling-circle DNA replication and they pose interesting questions as to how the network is formed and how it is resolved for packaging into progeny virions".

An analysis of the various shapes of HSV-1 DNA molecules isolated from nuclei of infected cells revealed viral DNA molecules that had circular-linear shapes (Friedman and Becker, 1977). These viral DNA molecules seem to indicate that intramolecular recombination events take place during viral DNA replication since circular and 8-shaped DNA molecules as well as circular-linear HSV-1 DNA molecules were identified. The small circular component of the 8-shaped DNA molecules was 8 nm long and the large linear component was 45 nm long corresponding the exact length of the U_S and U_L DNA components of HSV-1 DNA, respectively. A model for intramolecular recombination of HSV-1 DNA was reported by Becker (1978). It is possible that the „a" nucleotide sequence present between the TR_L and TR_S or nucleotide sequences in its vicinity may be involved in the intramolecular recombination events in HSV-1 and MDV-1 and other herpesviruses with the U_L and U_S segments of the viral DNA. Experiments to study the molecular and enzymatic processes of recombination and cleavage of the viral genomes reported by Dutch *et al.* (1995) showed that HSV-1 DNA replication is specifically required for high frequency homologous recombination between repeated sequences. The frequency of recombination between repeats of the U_L-DR1 region, previously identified as the only segment of the HSV-1 a-sequence indispensable for enhanced a-sequence recombination, was not significantly higher than that measured for other short sequences. Martin and Weber (1996) reported that the „a" sequence is dispensable for isomerization of the HSV-1 genome. These studies indicate that the molecular mechanisms of HSV-1 DNA replication and recombination and the definition of the conditions for the selection of ORI_L or ORI_S for the replication of HSV-1 genomes and the amplifications of genomic sequences in MDV-1 and HSV-1 are not fully understood. Table 5 lists changes in MDV-1 and HSV-1 genomes that emerged during replication or recombination of the viral DNA molecules.

The recombination events that occur during viral DNA replication in the infected nuclei may cause changes in the viral genome. A recombinant virus, HSV-1 (R15), was isolated from an experimental recombination event that inserted *Bam*HI fragment B from HSV-1 (F) DNA into HSV-1 (HFEM) DNA that has a 4 kbp deletion in its *Bam*HI B DNA sequence. A recombinant designated HSV-1 (R15) was found to have genomic rearrangements near and away from the sites of *Bam*HI-B DNA recombination events in its genome. These changes may be explained by the model of intramolecular recombination that was reported twenty years ago (Becker, 1978).

Conclusions

The studies on MDV-1 and HSV-1 and the availability of the complete nucleotide sequences of many herpesviruses provided the basis for the study of the herpesvirus evolution. Although the ancestral virus is not known, it is possible to understand the role of the intragenomic and extragenomic DNA recombination events during the adaptation of a herpesvirus to replicate in a new host. Studies are needed to address the question of how the MDV-1 DNA genome acquired the proper promoters of the eight blocks of herpesvirus genes for the correct expression in chicken cells, and how HSV-1 acquired cellular genes from the host genome, nuclear retrons or retroelements. The studies on the DNA genomes of MDV-1 and HSV-1 may indicate that the viral genomes continually change during their replication and recombination in infected cells *in vivo* or *in vitro* to yield new virus isolates that may have altered their ability to cause disease. The practical approach of the MDV-1 researchers that led to the development of protective vaccines within 2–3 years after the discovery of the MDV-1, long before the molecular biology of the virus was deciphered, should be adapted to the comprehensive knowledge on HSV-1 DNA genome and genes, in order to develop a safe vaccine to protect humans against HSV-1 infections. It is hoped that an effective vaccine to prevent HSV-1 infections and latency in humans will soon be available.

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