Identification of novel transcripts complementary to the Marek’s disease virus homologue of the ICP4 gene of herpes simplex virus

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Libraries of cDNA were generated from polyadenylated RNAs derived from Marek’s disease virus (MDV)-transformed cell lines by directional cloning of oligo-(dT)-primed cDNAs in lambda gt22A. Analysis of the libraries for viral sequences showed that a number of cDNA clones originated from transcripts mapping in the BamHI A region of the MDV genome. Sequencing and fine mapping of these cDNAs suggested that the RNA transcripts expressed from this region were either in the sense or antisense direction with respect to the MDV homologue of the ICP4 gene of herpes simplex virus. The longest cDNA clone from antisense transcripts was 2756 bp long and partially overlapped the 5’ end of the coding region of the ICP4 gene. The cDNA clone contained at least four introns, shown by comparison of its sequence with the sequence of the ICP4 gene. The presence of introns was confirmed by PCR analysis. All the introns have the consensus splice donor and acceptor signals at their 5’ and 3’ ends respectively. Northern blot analysis showed that the ICP4 gene homologue of MDV was abundantly transcribed only in lytically infected fibroblasts, whereas transcripts complementary to the ICP4 gene were the major transcripts in MDV-transformed cell lines and lymphomas. The transcripts complementary to ICP4 consist of two major RNA species approximately 15 kb and 1.32 kb long. The results suggest that there might be an inverse relationship between the abundance of ICP4 transcripts and their complementary transcripts in MDV-infected and transformed cells.

Introduction

Marek’s disease is a lymphoproliferative disease of chickens characterized by malignant transformation of CD4+ helper T lymphocytes. The disease is caused by a herpesvirus which has been classified as a gammaherpesvirus together with Epstein–Barr virus (EBV) on the basis of its biological properties. However, the genomic structure of Marek’s disease virus (MDV) and the organization of genes in the genome indicate a closer relationship to alphaherpesviruses such as herpes simplex virus (HSV) (Cebrian et al., 1982; Buckmaster et al., 1988; Velicer & Brunovskis, 1992).

Many cell lines have been established from Marek’s disease lymphomas but in vitro transformation is not easily achieved (Calnek & Schat, 1991). Cell lines contain multiple copies of viral DNA (Ross et al., 1981; Rziha & Bauer, 1982) and vary in the degree of expression of viral antigens and in their capacity to produce infectious virus when co-cultivated with permissive chicken kidney cells or fibroblasts. Previous studies have shown that viral gene expression in many cell lines is mainly restricted to the repeat regions of the genome (Silver et al., 1979; Schat et al., 1989; Sugaya et al., 1990). The majority of the transcripts have been shown to be immediate early (IE) transcripts (Silver et al., 1979; Schat et al., 1989; Sugaya et al., 1990), indicating that IE genes could have a significant role in maintenance of latency and of the transformed state. The main objective of the present work was to characterize the viral genes that are transcribed in cell lines and the products they encode. Libraries of cDNA have been derived from the producer cell line MSB1 and also from the non-producer cell line RPL1. The latter is of particular interest since it originated as a transplantable tumour which was subsequently adapted to growth in vitro (Nazerian et al., 1977). Infectious virus cannot be rescued from this line and viral gene expression is minimal. Consequently, the viral genes transcribed in RPL1 cells are more likely to be relevant to latency and transformation than those in producer cell lines. We report here on the identification of novel antisense transcripts which partially overlap the 5’ end of the MDV homologue of the major regulatory gene ICP4 of HSV. The antisense transcripts were predominantly expressed in MDV-transformed cells whereas the ICP4 sense transcript was predominant in
lytic infection. The results are consistent with the idea that transcription of antisense RNA could play a role in the regulation of gene expression in cell lines and in latency.

Methods

Cell lines and cell culture. MSB1 (Akiyama & Kato, 1974) and RPL1 (Nazerian et al., 1977) cell lines were cultured at 38 °C as previously described (Ross et al., 1981). Immunofluorescence tests using monoclonal antibodies specific for pp38 (Nakajima et al., 1987) and gB (Ross et al., 1989) showed that both antigens were expressed in 10% of MSB1 cell lines. In contrast, only 0.8% of RPL1 cells expressed pp38 and gB was not expressed (C. L. Jiang, personal communication). Chick embryo fibroblast (CEF) cells were cultured in roller bottles as previously described (Ross et al., 1975).

Virus and isolation of viral DNA. The HPR316 strain of MDV (clone 377) was grown in CEF cells as previously described (Ross et al., 1975). Viral DNA derived from purified virions or by Hirt extraction of infected CEF cells was prepared as described previously (Ross et al., 1981). BamHI fragments of MDV DNA were derived from our MDV cosmid library (data not shown) or from the library provided by Dr M. Noneyama (Showa University Research Institute for Biomedicine, St Petersburg, Fla., U.S.A.). Restriction enzyme digests of cloned DNA were separated by electrophoresis in agarose gels and individual fragments were purified using GENECLEAN (Bio 101) or QIAGEN columns (QIAGEN) according to the instructions of the manufacturers. Similar procedures were used to recover MDV cDNA inserts from recombinant lambda phage.

Construction of a cDNA library. Total RNA was extracted from approximately 10^8 MSB1 or RPL1 cells by the guanidinium thiocyanate (GTC)-CsCl method except that GTC was buffered with 10 mm-Tris–HCl pH 7.5 (Ausubel et al., 1989). mRNA was isolated using oligo(dT)-cellulose (type 7; Pharmacia). cDNAs from MSB1 and RPL1 cells were prepared and cloned into NorI and SalI sites of lambda gt22A (Superscript lambda system; BRL). Briefly, the first cDNA strand was synthesized using a NorI–oligo(dT) primer adaptor. After the second strand had been synthesized, a SalI adaptor was ligated to the cDNA which was then digested with NorI to produce a NorI site at the 3' end. The cDNA was then ligated to NorI and SalI arms of lambda gt22A.

Purification of lambda phage DNA. Recombinant lambda phage was propagated in *Escherichia coli* strain Y1090, essentially as described by the supplier (Promega). Briefly, *E. coli* Y1090 was grown in NZY broth supplemented with 10 mm-MgSO_4_ and 100 μg/ml of ampicillin at 37 °C in an orbital shaker for 2 h. Cultures (with an optical density at 600 nm of 0.4) were inoculated with lambda phage (greater than 10^9 p.f.u./ml) and incubated at 37 °C with continuous shaking for 4 to 6 h until complete lysis occurred. Purification of lambda DNA was carried out using the modified zinc chloride method (Santos, 1991). Briefly, 50 ml of culture supernatant was treated with 100 μl of DNase and RNase solution (20 mg/ml RNase A, 6 mg/ml DNase I, 0.2 mg/ml BSA, 10 mm-EDTA, 100 mm-Tris–HCl and 300 mm-NaCl, pH 7.5) at 37 °C for 30 min. One ml of 2 M-zinc chloride (BDH) was added to the culture supernatant and the mixture was incubated for 5 min at 37 °C. After centrifugation at 4000 r.p.m. for 20 min, the pellet obtained was resuspended in 5 ml of TES buffer (0.1 M-Tris–HCl pH 8.0, 0.1 M-EDTA and 0.3% SDS) and incubated at 37 °C for 15 min. Six-hundred μl of 3 M-potassium acetate solution (pH 5.2) was added and the mixture was kept on ice for 10 to 15 min. After centrifugation at 15000 r.p.m. for 10 min, the supernatant was collected and the phage DNA was purified using a lambda QIAGEN column (QIAGEN).

Dot blot and Southern blot hybridization. Southern blot hybridization and plaque and colony blotting using Hybrid-N membrane (Amersham) were carried out using conventional methods (Sambrook et al., 1989). Southern blot transfers were performed using a positive pressure blotter (PosiBlot; Stratagene). DNA probes for hybridization were labelled either with [α-32P]dCTP by random primer extension (Mega-prime system, Amersham) or with a non-radioactive marker (ECL direct nucleic acid labelling and detection system; Amersham). Washing of blots hybridized to 32P-labelled probes was carried out under high stringency conditions (0.1 × SSC, 0.1% SDS) at 55 °C for 30 min. In the case of the ECL system, prehybridization and hybridization were conducted at 42 °C using ECL gold buffer (Amersham). Different washing conditions were used, ranging from 5 to 30 min at room temperature (low stringency wash) to 55 °C (high stringency). Bound probe was detected using the ECL nucleic acid detection reagent (Amersham).

Screening of cDNA libraries. Plaques produced by recombinant lambda were blotted onto Hybrid-N membrane (Amersham). Initially, plaque lifts were hybridized to a MDV DNA probe. Individual positive plaques were picked and the plaque was eluted from the agar by incubation in SM buffer (50 mm-Tris–HCl pH 7.5, 100 mm-NaCl, 8 mm-MgSO_4_ and 0.001% gelatin) containing chloroform. The process of plaque purification was repeated until 100% of the plaques were positive. To map the cDNA clones in the MDV genome, the cloned cDNA inserts were separated from lambda DNA, labelled with 32P (Megaprime system; Amersham) and used as probes for hybridization to Southern blots of restriction enzyme digests of MDV DNA.

Subcloning and sequencing, cDNA inserts were subcloned into pBluescript which had been digested with NorI and SalI and recombinant plasmids were used to transform *E. coli* strain XL1-Blue (Stratagene) using conventional methods (Sambrook et al., 1989). Purified plasmid DNA (QIAGEN columns) was used for sequencing and other analysis. Two sets of oppositely orientated, nested deletions were prepared from plasmid DNA using the Nested Deletion Kit (Pharmacia) and were sequenced using modified T7 DNA polymerase (Sequenase 2.0, USB) or Taq polymerase (dsDNA cycle sequencing system, Gibco). Sequence data were assembled using DNASIS software.
Transcripts complementary to MDV ICP4

Fig. 2. Physical mapping of M42 and M49 cDNA clones in the MDV genome by Southern blot hybridization. (a) BamHI digests of MDV DNA were hybridized to MDV DNA (lane 1), M42 cDNA (lane 2) and M49 cDNA (lane 3). The position of the BamHI A fragment and a 15 kb fragment (see text), are indicated. (b) Hybridization of M49 and M42 clones to restriction enzyme digests of the BamHI A fragment of MDV (i). The enzymes used were BgII (B), EcoRI (E), HindIII (H), PstI (P), SmaI (S) and XhoI (X). The subfragments of BamHI A (Fukuchi et al., 1984a, b) that hybridized to M49 and M42 are represented graphically with shaded boxes in (ii).

Results

Mapping viral cDNA clones

Preliminary screening of the MSB1 library indicated that most cDNAs from MSB1 mapped to the BamHI H and
Fig. 3. For legend see opposite.
The nucleotide sequence of M49 cDNA. The ICP4 binding site, poly(A) signal and poly(A) tail are underlined. Splicing sites are indicated by vertical arrows. The portions of primers 1 and 3 are underlined and the direction of primer extension in PCR is indicated by dashed arrows. The sequence overlapping ICP4 is in bold letters (positions 1 to 2234). The location of the BstEII restriction enzyme site is indicated. Predicted aa sequences of the four potential ORFs are indicated below the nucleotide sequence.

A fragments of MDV (Fig. 1). In this paper we have focused on the cDNA clones mapping in the BamHI A fragment which spans the short internal repeat (IRs) and a large part of unique short (Us) region. Six clones mapping in this region were isolated. Southern blot hybridization (Fig. 2a) showed, as expected, that the cDNA clones also hybridized to another fragment (15 kb) which is likely to span the short terminal repeat (TRs).

To map the cDNA clones more precisely, the cDNAs were labelled with $^{32}$P and hybridized to Southern blots of restriction enzyme digests of the MDV BamHI A fragment (Fukuchi et al., 1984a). The results indicated that all of the clones mapped in the repeat region of BamHI A; five clones (M5, M7, M18, M33 and M42) mapped in the region of BamHI A proximal to the long internal repeat (IRs) and one clone (M49) mapped in the region of IRs adjacent to Us. In this paper we will refer only to clones M42 and M49 (Fig. 1 and 2).

Sequencing and sequence analysis of cDNA clones

Initially, partial sequencing of the 5' and 3' ends of the cDNAs showed that the sequence of M49 matched the complementary strand of ICP4, since its 5' end matched nucleotide 2234 of ICP4 in the sequence reported by Anderson et al. (1992) and its 3' end terminated beyond the start codon of ICP4 in a region that has not yet been sequenced. It was also noted that 87 nucleotides (2192 to 2106 of ICP4) were missing in the M49 cDNA but that sequences further downstream in the cDNA matched sequences towards the 5' end of ICP4. We concluded that clone M49 had originated from a transcript running in an antisense direction with respect to ICP4 and that it was probably spliced (Fig. 1).

The entire sequence of clone M49 is shown in Fig. 3. The cDNA clone is 2756 nucleotides long, has an overall G and C content of 54.4% compared to 51.7% for the MDV ICP4 homologue and overlaps a significant part of the 5' end of the coding region of ICP4. When the nucleotide sequence of M49 was compared to that of the ICP4 gene of MDV, it became evident that M49 cDNA had originated from a highly spliced transcript and had at least four introns within the region that overlaps the MDV ICP4 gene (positions 2106 to 2192, 1496 to 1707, 985 to 1069 and sequences preceding position 908 in the ICP4 sequence; Anderson et al., 1992). The fourth intron (nearest to 3' end of antisense transcript) is located upstream of the coding region of MDV ICP4 at nucleotide 908 and extends into a region of the MDV genome which has not yet been sequenced (Fig. 1). Consequently we are not able to draw any conclusions about splicing at the 3' end of the antisense transcript.

The nucleotide sequence of the exons of M49 that overlapped the 5' end of ICP4 showed at least 98% identity with the complement of ICP4 (Anderson et al., 1992). This suggests that the deletions in M49 which were deduced by comparison of its sequence with the sequence of the ICP4 gene of the GA strain of MDV (Anderson et al., 1992) are due to splicing rather than to virus strain differences. Inspection of the MDV ICP4 genomic sequence and of the M49 sequence revealed the presence of consensus splice donor and acceptor signals at the 5'
Table 1. Splice donor and acceptor sequences in the ICP4 gene

<table>
<thead>
<tr>
<th>Intron</th>
<th>Donor sequence</th>
<th>Acceptor sequence</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>ATCCGTAAGT (2187-2196)†</td>
<td>GCAGAGGTC (2101-2109)</td>
</tr>
<tr>
<td>2</td>
<td>TCCGGTGAGT (1702-1711)</td>
<td>CCAGAACGA (1491-1499)</td>
</tr>
<tr>
<td>3</td>
<td>CCAGGTATTA (1064-1073)</td>
<td>GCAGGCATT (981-988)</td>
</tr>
<tr>
<td>4</td>
<td>CTCGGTGAGT (903-912)</td>
<td>ND‡</td>
</tr>
</tbody>
</table>

* Arrows indicate splicing sites either at 5' end (donor) or 3' end (acceptor) of the introns.
† Sequences (with nucleotide positions) cited are complementary to the sequence of ICP4 published by Anderson et al. (1992).
‡ ND, Sequence not determined.

![PCR analysis of genomic DNA from MSB1 cells and MDV DNA from lytically infected CEF cells.](image)

Fig. 4. PCR analysis of genomic DNA from MSB1 cells and MDV DNA from lytically infected CEF cells. PCR products in lanes 1 to 4 were generated using primers 1 and 3 and those in lanes 5 to 8 were obtained using primers 1 and 2. Templates for PCR were: lanes 1 and 5, M49 cDNA; lanes 2 and 6, MSB1 DNA; lanes 3 and 7, MDV DNA; lanes 4 and 8, attenuated MDV DNA. Fragment sizes in kbp were estimated using a 1 kbp DNA ladder (lanes M).

We have also noted that the cDNA clone M49 contained the sequence ATCGTCCATATTGGC (positions 547 to 561; Fig. 3) which agrees with the consensus sequence (ATCGTCNNNNYCGRC) that functions as a binding site of the ICP4 protein of HSV (Faber & Wilcox, 1986). A similar, though less conserved, sequence has also been reported upstream of the ICP4 gene of MDV (Anderson et al., 1992). It is therefore possible that transcription of the antisense RNA could also be regulated by binding of the MDV ICP4 protein.

A search for coding regions in M49 revealed a number of small potential open reading frames (ORFs), the largest encoding 150 amino acids (aa) (positions 426 to 876) and which overlapped the coding region of ICP4 (Fig. 3). However, comparison of the predicted aa sequence to the sequence of proteins in databases did not reveal any convincing similarity to known proteins.

The 5' end of M42 matched nucleotide position 2863 of the ICP4 gene (Anderson et al., 1992) and its 3' end extended to nucleotide 5546 of ICP4, only 17 nucleotides upstream of the poly(A) signal (AATAAA). The size of M42 (28 kbp), determined by electrophoresis in agarose gels, was consistent with the expected size (2863 bp) calculated from the ICP4 sequence (Anderson et al., 1992). We concluded that, in contrast to M49, M42 originated from sense transcripts of the ICP4 gene and that it was probably not spliced (Fig. 1).

**PCR analysis of cDNA clone M49 and genomic DNA from the MSB1 cell line**

Our evidence for splicing of the antisense transcript has been based on sequence differences between the cDNA clone M49 and the ICP4 gene of MDV. In view of the observation that MSB1 cells are transformed by the BCI strain of MDV (Akiyama & Kato, 1974), whereas the ICP4 sequence is that of the GA strain (Anderson et al., 1992), it could be argued that the sequence differences are attributable to the virus strains used and not to splicing. To confirm that M49 had been generated from a spliced transcript, we analysed the cDNA clone M49 and genomic DNA from MSB1 cells by PCR.

Three oligonucleotide primers, depicted in Fig. 1 and 3, were used for the PCR reactions. Primer 1 (5' GATCGCCACCCACCGATTACTACCT 3') is from the second exon (nucleotides 242 to 265; Fig. 3). Primer 2 (5' AATGAGCGAACCTGCTCACACAAC 3') is from...
Identification of transcripts complementary to the ICP4 gene in MDV-infected and transformed cells by Northern blot hybridization

The identification of ICP4 antisense transcripts in this study has been based so far on the results of sequencing cDNAs from cell lines. To estimate the size of the transcripts and to determine whether they are also present in MDV-infected CEF cells and lymphomas, we have analysed RNAs obtained from these sources by Northern blot hybridization. The results of hybridization with a riboprobe generated with T7 RNA polymerase, which recognizes ICP4 antisense transcripts, are shown in Fig. 5(a). Two major RNAs (of approximately 15 kb and 1-32 kb), which varied in relative abundance in the cells examined, were detected. The two cell lines, particularly the non-producer cell line RPL1, expressed large amounts of the small RNA. Interestingly, lymphoma tissue contained only the small RNA species and this was present only at low levels [Fig. 5(a) lanes 4 and 8]. We have also noted the presence of two minor RNAs (4-1 kb and 2-4 kb) in the mRNA preparation from RPL1 cells which could be intermediate RNA species of virulent and attenuated strains of MDV in the region of IR₈ examined in this study.

Identification of transcripts complementary to MDV ICP4

Transcripts complementary to MDV ICP4 1719

Fig. 5. Northern blot analysis of MDV-infected and transformed cells for ICP4 sense and antisense transcripts. (a) Hybridization to a riboprobe driven by T7 polymerase to detect antisense transcripts. Lanes 1 to 5 contain total RNAs (about 20 μg/lane) extracted from MDV-infected CEF cells, MSB1 cells, RPL1 cells, MDV-induced lymphomas and mock-infected CEF cells respectively. Lanes 6 to 8 contain mRNAs (5 μg/lane) isolated from MSB1 cells, RPL1 cells and MDV-induced lymphomas respectively. Parts (i) and (ii) show autoradiographs obtained after short and long exposure times respectively. (b) Hybridization to a riboprobe driven by T3 polymerase to detect ICP4 sense transcripts. Lanes 1 to 4 contain total RNAs extracted from MDV-infected CEF cells, MSB1 cells, RPL1 cells and MDV-induced lymphomas respectively. An RNA ladder (BRL) was used to estimate the size of the RNA bands as indicated (kb). The positions of chicken ribosomal RNAs (r1 and r2) are indicated.

the second intron (nucleotides 1578 to 1651 in the ICP4 sequence; Anderson et al., 1992) and runs in the opposite direction with respect to primer 1. Primer 3 (5' TTTCACCATACGCCAGCCACTC 3') is from the antisense sequence (nucleotides 1806 to 1827; Fig. 3) and is upstream of the coding sequence of ICP4. This primer is also in the opposite orientation to primer 1 (Fig. 1 and 3). Primer pairs 1 and 2 or 1 and 3 were used to amplify DNA from the cDNA clone M49, genomic DNA from MSB1 cells and viral DNA from infected CEF cells. The results (Fig. 4) showed that no amplified product was obtained using M49 cDNA as a template and the primer combination 1 and 2 (lane 5). This confirmed that primer 2 is located in an intron. In contrast, primer pair 1 and 2 amplified a product of the expected size (0-3 kb) when genomic DNA from MSB1 or viral DNA was used as the template (lanes 6 and 7). Moreover, the primer pair 1 and 3 amplified a 1-6 kb fragment using M49 as template (lane 1), in agreement with the expected size (1587 bp) deduced from the cDNA sequence, but amplified a 3-5 kb fragment when genomic DNA from MSB1 (lane 2) or viral DNA (lane 3) was used as template. These results confirmed the presence of introns in the cDNA clone M49 and further indicated that there was no major deletion or expansion between the sequence of genomic DNA derived from MSB1 cells and genomic DNA from virulent and attenuated strains of MDV in the region of IR₈ examined in this study.
low abundance. Lytically infected CEF cells expressed relatively small amounts of the antisense transcript. None of these RNAs was detected in mock-infected CEF cells.

The results of hybridization to the riboprobe generated with T3 RNA polymerase, which recognizes ICP4 sense transcripts, are shown in Fig. 5(b). A large RNA transcript, approximately 14 kb long, was detected only in lytically infected CEF cells. No bands were seen in RNA extracts from cell lines and lymphomas even on longer exposure (data not shown). This suggests that expression of ICP4 in these cells is minimal compared to lytically infected CEF cells.

Discussion

The precise role of MDV in transformation of T lymphocytes is not known. There is evidence that the virus is latent in primary lymphomas and in the majority of the cells of several cell lines (Calnek et al., 1981; Rziha & Bauer, 1982; Hirai et al., 1986; Delecluse et al., 1993). In this study we have sought to uncover the viral genes that are transcribed in cell lines and have prepared cDNA libraries from the expression cell line MSB1 and also from the cell line RPL1 in which virus expression is repressed. We expect that many of the genes expressed in MSB1 could be superfluous to the establishment or maintenance of transformation and that viral transcription in RPL1 would be more similar to expression in primary lymphomas. This is supported by the observation that 10% of the MSB1 cells expressed gB and pp38 antigens whereas less than 0.1% of RPL1 cells expressed pp38, and gB was not detected.

The main finding of this study is the identification of novel transcripts complementary to the MDV homologue of the ICP4 gene of HSV, which were abundant in the RPL1 cell line but less so in MSB1 cells. Comparison of the sequence of the M49 cDNA to that of the ICP4 gene of MDV (Anderson et al., 1992) suggested that the antisense transcript was highly spliced and that at least four exons, representing 946 bases, overlapped the coding region of MDV ICP4. Since the published sequence of MDV ICP4 was from a different strain of MDV than that which transformed MSB1 (BC1 strain) or RPL1 (JM strain), it was necessary to examine the possibility that the apparent deletions noted in the cDNA with respect to the sequence of ICP4 were not attributable simply to virus strain differences. To that end we carried out PCR analysis of genomic DNA from MSB1 cells and the cDNA clone M49, using primers mapping in the introns and exons of the antisense transcript. We also examined viral DNA from the HPRS16 and HPRS16/att strains. The results (Fig. 4) allowed us to conclude that the antisense transcript was indeed spliced and also indicated that attenuation of MDV did not result in major deletions or expansions in this region of the genome. In the case of HSV, it has been shown that splicing of the latency-associated transcripts (LAT) occurs even though the 5' end of the intron does not conform to the consensus splice donor signal GT (Spivack et al., 1991). Here we have shown that the 5' ends of all the introns that we deduced by comparison of the cDNA to genomic DNA sequences conform to the consensus splice donor signal (Mount, 1982). This is further evidence of splicing in our system.

The Northern blot hybridization confirmed the presence of transcripts complementary to ICP4 in MDV-infected and transformed cells. The results also showed that there are two major transcripts (of approximately 15 kb and 1.32 kb) which were more abundant in RPL1 than in MSB1 cells. This is consistent with the observation that the frequency of cDNA clones derived from antisense RNA was higher in the RPL1 library than in the MSB1 library. It is interesting that both antisense transcripts were also present, albeit in small amounts, in lytically infected CEF cells. The specificity of hybridization was confirmed using a riboprobe that hybridizes to ICP4 transcripts. In this case one major RNA transcript was detected in lytically infected CEF cells but not in transformed cell lines or lymphomas. These findings suggest that transcripts complementary to the MDV ICP4, which probably functions as a major regulatory gene in MDV replication, could have a role in suppressing viral gene expression and in latency. The relationship between the 15 kb and 1.32 kb antisense RNAs is unknown. But since both RNAs shared the same sequence, it is likely that the small RNA is processed from the large one. Some minor mRNA species noted in the RPL1 mRNA preparation may be intermediates in the processing of the large RNA.

RNA transcription in cells latently infected with herpesviruses has been extensively studied. In the case of HSV-1, transcription during latency is apparently limited to a single transcription unit complementary to ICP0, partially overlapping its 3' domain (Rock et al., 1987; Stevens et al., 1987; Goridon et al., 1988). The transcription unit designated as a LAT is transcribed first as an unstable large RNA (8.3 kb) and is subsequently cleaved into 20 kb, 1.5 kb and 1.45 kb RNAs (Goridon et al., 1988). Small RNAs may be derived from larger ones by splicing (Wagner et al., 1988; Spivack et al., 1991). The function of the LATs in HSV latency is not clear. In the EBV-associated tumour nasopharyngeal carcinoma, a transcription unit (apparent sizes of 4.8, 5.2, 6.2 and 7.0 kb) of abundant and highly spliced polyadenylated RNAs complementary to several genes required for virus replication (such as DNA polymerase) has been reported (Hitt et al., 1989; Gilligan et al., 1990;
Karran et al., 1992; Brooks et al., 1993; Zhang et al., 1993; Smith et al., 1993). It has been postulated that these transcripts could play a role in the maintenance of latency by interfering with translation of the genes required for virus replication. In the case of pseudorabies virus (PRV), several non-polyadenylated RNAs, transcribed from the strand complementary to the PRV homologue of HSV ICP4, have been detected in latently-infected cells. The transcribed region extends from U₉ to IR₈ and is at least 11·8 kb long (Priola et al., 1990; Priola & Stevens, 1991). In MDV-infected CEF cells, multiple bidirectional transcripts have been detected in the IR₈ region corresponding to ICP0 of HSV (Chen & Velicer, 1991). However, the proteins of MDV are not homologous to ICP0 of HSV. So far it appears that MDV and PRV are the only examples where antisense transcription of ICP4 has been reported.

The antisense transcripts of ICP4 could have two functions in MDV-transformed cells. They could interfere with virus replication by blocking translation of ICP4 or they could code for oncoproteins that are associated with cell transformation. Inhibition of the lytic cycle of the virus can be regarded as an essential step in the maintenance of the transformed state and there is evidence that methylation of viral DNA may contribute to this in cell lines (Kanamori et al., 1987) but to a lesser extent in primary lymphomas (L. J. N. Ross, unpublished results). The observation that ICP4 sense transcripts were predominantly expressed in lytic infection and antisense transcripts were predominantly produced in MDV-transformed cells (latent infection) is consistent with the marked differences in the degree of expression of viral antigens in these two systems. It is possible therefore that there might be a correlation between the relative abundance of sense and antisense transcripts and latency in MDV-transformed cell lines. Further work is required to determine the function of the complementary transcripts and to identify the proteins they may encode.

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References


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