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THE 132 bp REPEATS ARE PRESENT IN RNA TRANSCRIPTS FROM 1.8 kb GENE FAMILY OF MAREK DISEASE VIRUS-TRANSFORMED CELLS

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Summary. - Marek's disease virus (MDV) is an oncogenic, lymphotropic herpesvirus of chickens. Loss of its tumourigenic potential is believed to be associated with amplification of the 132 bp repeats from *Bam*HI-D and *Bam*HI-H fragments. We prepared cDNA libraries from RPL1 and MSB1 cell line and from the latter we identified a clone which spanned the 132 bp repeats within the *Bam*HI-H fragment. By sequencing and Northern blot analysis we confirmed the presence of the 132 bp repeats. The analysis by PCR made on the total RNA revealed two 132 bp repeats in MDV transcripts from RPL1 and two to three repeats in transcripts from MSB1 cells. These results show that sequences within the 132 bp repeats are transcribed and are not spliced out as previously reported.

Key words: Marek disease virus; MSB1, RPL1 transformed cells; RNA transcripts; 132 bp repeats

MDV is an avian herpesvirus which causes malignant lymphomas in chickens (Churchill and Biggs, 1967). The mechanism by which it induces neoplasia is still unclear. It was found that serial passage of MDV in cultured cells resulted in loss of its oncogenicity, which appears to be correlated with an expansion of the *Bam*HI-H- and D-fragments localized in the IR_L and TR_L regions (Maotani *et al.*, 1986). This expansion was shown to be due to the amplification of the 132 bp direct repeats found within the *Bam*HI-D- and H-fragments.

In the MDV-transformed cell line MSB1 (Bradley *et al.*, 1989*a*) several species of 1.8 kb long RNAs were identified in which the 132 bp repeat region was spliced out. It also was reported that the loss of MDV tumourigenicity was accompanied by a truncation of this 1.8 kb transcript (Bradley *et al.*, 1989 *b*). However, in chick embryo fibroblasts (CEF) infected with oncogenic and non-oncogenic MDV, Iwata *et al.* (1992) detected RNA transcripts containing the 132 bp repeats.

There are some discrepancies in the reported transcription of regions flanking the 132 bp repeats. It is not known, whether these sequences have the capacity to

code for proteins, as they contain only two short ORFs. The view that 1.8 kb RNA transcripts might play a role in the oncogenicity of MDV was supported by the experiments performed with antisense oligonucleotides complementary to these transcripts which were able to inhibit the proliferation of MDV-transformed MSB1 cells (Kavamura *et al.*, 1991).

In this report we describe a cDNA clone carrying the 132 repeat region which we identified in our cDNA library prepared from MSB1 cell line.

Total RNA from MSB1 and RPL1 cell lines was prepared (Chirgwin *et al.*, 1979) and mRNA was separated on oligo-dT cellulose. cDNA was synthesized using SuperScript cDNA System (BRL), using *Eco*RI-*Not*I adaptors (Stratagene). It was ligated into lambda gtll (Promega) and *in vitro* packaging was performed with Gigapack Gold (Stratagene).

Amplified cDNA library was plated on *E. coli* Y 1090 cells and phages were transferred onto Hybond N membrane (Amersham). Probes used for hybridization were MDV HPRS 16 DNA, or *Bam*HI-H fragment which were labelled with ^{32}P-alpha-dCTP by random priming (Multiprime labelling DNA system, Amersham). Hybridization was carried out under stringent conditions at 42 °C in the presence of 50 % formamide. Final wash was at 65 °C in 0.1 ×SSPE and 0.1 % SDS. Clones positive after three rounds of rescreening were picked up and their inserts were cloned into *Not*I site of pBluescript II KS.

MDV genomic DNA digested by *Bam*HI was separated in 0.8 % agarose gel in TBE buffer and alkali transferred onto Hybond N membrane. The blot was hybridized with the cDNA insert under conditions described above.

cDNA insert ME1 or whole lambda gt11 c DNA libraries (from MSB1 and RPL1) were used as templates for PCR with primers R1 (5' ATGTATGTGTGGGAGAAA 3') and R2 (5' TGTAATATAAGGGCACCT 3') and *Taq* polymerase (Promega). PCR was performed in 30 cycles at 94 °C (1 min), 45 °C (1 min) and 72 °C (1 min).

Total cellular DNA was extracted with proteinase K - sodium dodecyl sulphate followed by phenol extraction. 1 μg of DNA was used as a template for PCR under the same conditions as described above.

PCR of RNA was performed according to the method of Kawasaki (1990). First strand cDNA synthesis was done by Superscript reverse transcriptase (BRL) with random hexamers as primers. PCR was performed with R1 and R2 primers under the same conditions as described above.

Nested deletions in two opposite directions were prepared using the Erase-a-Base kit (Promega) and alkali denaturated dsDNA was sequenced by dideoxy-chain-termination method using the T7 sequencing kit (Pharmacia).

15 μg of total RNA from MSB1 was separated on 1.2 % gel in the presence of 2.2 mol/l formaldehyde and blotted onto Hybond C Super membrane (Amersham). 602 bp PCR product containing three 132 bp repeats was labelled with ^{32}P-alpha-dCTP by random priming. Hybridization conditions were the same as described above. 0.24–9.5 kb RNA ladder (BRL) was used as a size standard.

In order to identify genes expressed in MDV-transformed cell lines MSB1 and

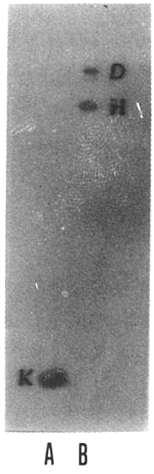

Fig. 1
Southern blot analysis
(A): of 470 bp PCR product from *Bam*HI-H fragment from MDV, strain HPRS16, containing two 132 bp repeats (control); (B): *Bam*HI digest of MDV DNA strain HPRS16 hybridized with ^{32}P-labelled clone ME1. D, H - *Bam*HI-D and -H fragments; K - control.

RPL1 we prepared cDNA libraries from these cells. After the screening of MSB1 cDNA library with MDV genomic DNA and *Bam*HI-H fragment we obtained a number of lambda gt11 positive clones. Inserts of three of them were approximately 1.2 kb in size and were specific for *Bam*HI-H- and D-region (Fig. 1). As these regions are believed to be associated with the tumourigenic potential of MDV, we decided to study them further. ME1 clone insert was subcloned into *Not*I site of pBluescript KS$^+$, sequenced and it was found out that this clone belonged to the 1.8 kb gene family. The sequencing results together with analysis by PCR revealed that it contained the 132 bp repeats and

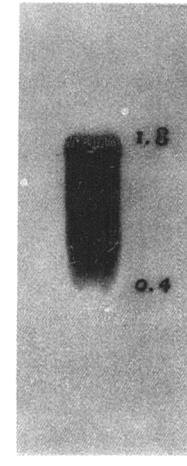

Fig. 2
Northern blot analysis
Total RNA from MSB1 cell line hybridized with ^{32}P-labelled 602 bp PCR product of ME1 clone containing three 132 bp repeats.

that this particular clone is not spliced. The sequence of ME1 is starting at position 697 and ending at 1740 of *Eco*RI-*Bam*HI clone from *Bam*HI-H fragment (Bradley *et al.*, 1989*a*). The ME1 clone contains three 132 bp repeats. This finding is not consistent with the results obtained by Bradley *et al.* (1989*a*) who reported, that the 1.8 kb mRNA extracted from the IdU-treated MDV-transformed tumour cell line MCB1 was composed of 2 exons, located on both sites of the 132 bp repeats. However, RNA transcripts similar to ours (as to the presence of the 132 bp repeats) were found also by Iwata *et al.* (1992) in CEF cells infected by oncogenic as well as non-oncogenic MDV. The fact, that 1.8 kb gene family contains spliced and non-spliced transcripts was supported by the results obtained by Peng *et al.* (1992), who identified both types of transcripts in cDNA library constructed from CEF infected with RB1B oncogenic strain of MDV. Our findings were confirmed also by Nothern blot analysis of total MSB1 RNA with 602 bp probe containing three 132 bp repeats (this probe was obtained by PCR amplification of ME1 clone using R1 and R2 primers flanking repeats). We detected a smear of transcripts ranging from 0.4 to 1.8 kb, suggesting that all of them contained the 132 bp repeats (Fig. 2).

We analyzed PCR products obtained from the whole MSB1 and RPL1 DNA and total RNA. In the cell line MSB1 we detected transcripts containing 2 or 3 repeats both on DNA and RNA and in the case of RPL1 only 2 repeats (Fig. 3). These results were verified by Southern blot analysis using *Acc*I fragment within the 132 bp repeats as the probe for hybridization.

The function of the abundant 1.8 kb gene family RNA is unclear. There are two ORFs; ORF1 containing 63 aminoacids (aa) and ORF2 64 aa, when three 132

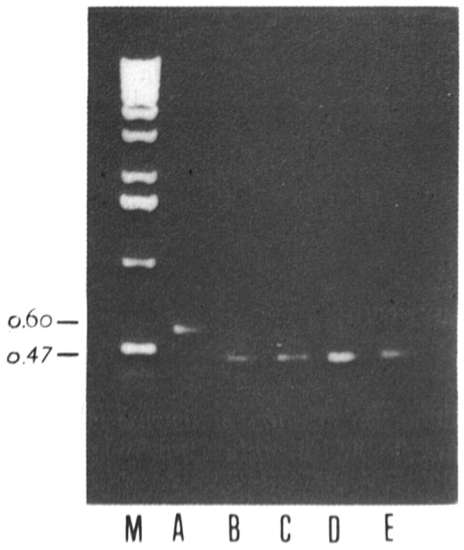

Fig. 3
Analysis of PCR products from genomic DNA and total RNA
(M) 1 kb ladder size markers, (A) PCR of ME1 clone with R1 and R2 primers, (B) PCR of 1 μg genomic DNA from MSB1 cell line, (C) PCR of 1 μg genomic DNA from RPL1 cell line, (D) PCR of total RNA from MSB1, (E) PCR of total RNA from RPL1.

bp repeats are present, ORF2 has coding capacity for 108 aa (including the third repeat region). Protein homology analysis using Prosis software (Hitachi Software Engineering) did not reveal any significant homology of ORF1 and ORF2 with proteins in SWISS-PORT library, release 21.0. It also remains to be determined whether the 132 bp repeats are related to the tumourgenicity of MDV or not.

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