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Circular Forms of Viral DNA in Marek's Disease Virus-Transformed Lymphoblastoid Cells

Brief Report

By

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With 3 Figures

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Summary

The state of viral DNA in three Marek's disease virus (MDV)-transformed chicken cell lines (MDCC-MSB-1, MDCC-HP-2, MDCC-RP-1) was investigated by CsCl-density gradient centrifugation, and sedimentation analysis in neutral glycerol gradients. Each cell line contained MDV DNA integrated into the host cell genome. Additionally, free viral DNA could be identified in MDCC-MSB-1 and MDCC-HP-2 cells, sedimenting at about 100 S, and banding at the position of circular DNA in CsCl-ethidiumbromide gradients. Thus, MDV DNA with properties of circular plasmid DNA could be demonstrated in 2 virus-transformed cell lines. The significance of circular plasmid forms of viral DNA remains yet to be clarified, since these are apparently not regularly found in MDV-transformed cells.

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MDV infection causes the development of lymphoid tumours in chickens. Apparently, an abortive viral infection of T-cells leads *in vivo* to malignant transformation, and results in a neoplastic disease (for review see 14). Several lymphoblastoid cell lines carrying T-cell markers have been established from such tumour tissues (13, 14, 15), and infectious virus can be rescued from some of those cell lines, with the exception of the virus-nonproducer lines (13, 16). MD lymphoblastoid cell lines contain multiple copies of the viral genome (9, 11, 12). In the virus-nonproducer line MKT-1 Tanaka *et al.* (16) found most of the viral DNA in a free unintegrated form showing properties of covalently closed supercoiled molecules. However, the studies of Kaschka-Dierich *et al.* (6) demonstrated in the low virus-producer line MDCC-HP-1 only an association of viral DNA with cellular DNA. In contrast, in the producer line MDCC-MSB-1, they found free and integrated MDV DNA, but failed to demonstrate the existence

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of MDV circular plasmids (6). Thus far, with regard to the state of MDV DNA in virus-transformed cells the reported data are not conclusive. In this communication we investigated 2 virus-nonproducer lines, MDCC-HP-2 (15) and MDCC-RP-1 (13), and in addition the moderate virus-producer line MDCC-MSB-1 (3). We show biochemical evidence for the presence of circular MDV DNA in MDCC-MSB-1- and MDCC-HP-2-cells.

The cells were grown at 41° C in RPMI-1640 medium supplemented with 10 per cent fetal calf serum, 10 per cent tryptose phosphate broth, and antibiotics. The JM strain of MDV was propagated in chicken embryo fibroblasts, and the viral DNA was prepared essentially according to Tanaka *et al.* (16). Actively growing cells of each of the 3 cell lines, containing more than 90 per cent living cells as determined by trypan exclusion tests, were concentrated to about 10^7 cells/ml. After gentle lysis of the cells the high mol. wt. DNA was fractionated by neutral CsCl-density gradients, and the viral DNA sequences were detected by DNA-cRNA hybridization as previously described by Lindahl *et al.* (10). Under these conditions, MDV DNA with a density of 1.705 g/ml (8) could be separated from the less dense cellular DNA in an artifical mixture of purified virion DNA and DNA of uninfected chicken fibroblasts (data not shown). Fig. 1 demonstrates the hybridization profiles of DNA from the 3 cell lines with MDV ^{3}H-cRNA after centrifugation in neutral CsCl-density gradients. Since the hybridization profiles of MDCC-MSB-1 and MDCC-HP-2 DNA were nearly identical, only one representative line is shown in the figure. Both cell lines exhibited a MDV DNA peak at the density position of free viral DNA (1.705 g/ml), in addition to viral DNA sequences in the density range of cellular DNA (Fig. 1a). Integrated viral DNA would be expected to band in CsCl-density gradients at a lower density than would free viral DNA. To test for an association of MDV DNA with less-dense cellular DNA, for each cell line the gradient fractions of cellular DNA density were combined. Half the amount of each was re-banded in a second CsCl-gradient, the other half was also re-centrifuged, but after reducing the size of the DNA by hydrodynamic shearing (1). As shown in Fig. 1c, some further free viral DNA could be separated from both, MDCC-MSB-1 and MDCC-HP-2 DNA, indicated by a small peak at the viral density. In MDCC-RP-1 cells, however, the majority of MDV DNA sequences was detected again in the cellular DNA range (Fig. 1d). In addition, a small peak of viral DNA was consistently found at a density position somewhat lower than that of free viral DNA (Fig. 1d, Fraction 18). This suggests the existence of hybrid molecules in the MDCC-RP-1 cells. After size reduction of the DNA, a large portion of the MDCC-MSB-1 and MDCC-HP-2 DNA, respectively, was released, and banded at the density of free DNA (Fig. 1e). However, only a small amount of the MDCC-RP-1 DNA could be shifted to a higher density after size reduction (Fig. 1f). Additionally, under denaturing conditions, MDV DNA could not be released from MDCC-RP-1 DNA, as tested by alkaline CsCl gradients (data not shown). Although these data may suggest that MDV DNA was mostly associated with the cell genome of the MDCC-RP-1 cells, whereas both integrated and free viral DNA were found in the MDCC-MSB-1 and MDCC-HP-2 lines, definitive evidence for integration has not yet been obtained. The mixture of virus and cells used as a control in our experiments did not exclude the possibility of the existence of free viral DNA

trapped by entanglement in cellular DNA. Furthermore, a possible density shift due to methylation of viral DNA in the transformed cells cannot be ruled out.

To investigate the sedimentation properties of the free viral DNA, the fractions of MDCC-MSB-1 and MDCC-HP-2 DNA enriched for MDV DNA by CsCl gradient centrifugation (Fig. 1a, Fraction 17—20) were further analysed by glycerol

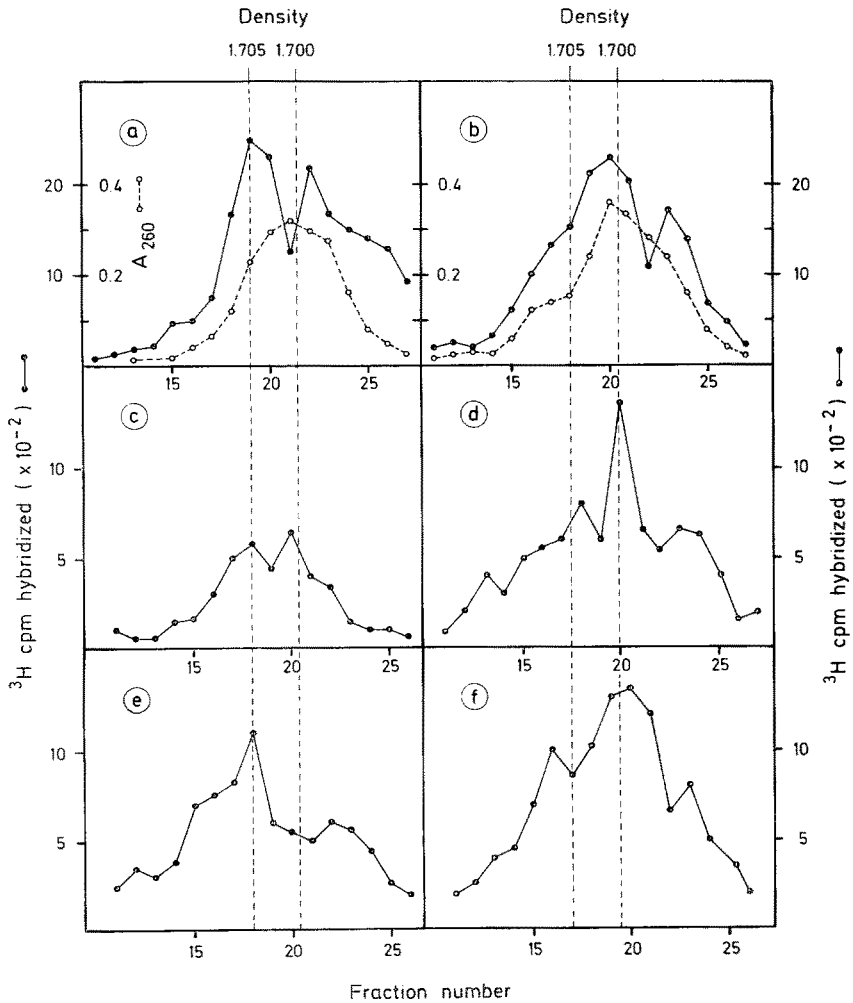

Fig. 1. Neutral CsCl-density gradient centrifugation of high M.W. DNA from *a* MDCC-MSB-1 and MDCC-HP-2 cells, and *b* MDCC-RP-1 cells. The gradients were run in a 50 Ti rotor at 33,000 rpm/min and 20° C for 65 hours. The fractions with densities of 1.695—1.702 were pooled, and each was divided into 2 parts. The M.W. of the DNA of one part of each was reduced by shear treatment. The DNA solutions were individually re-banded in CsCl under the same conditions as described above. *c* Unsheared DNA of MDCC-MSB-1 and MDCC-HP-2 cells, and *d* of MDCC-RP-1 cells. *e* Sheared MDCC-MSB-1 and MDCC-HP-2 DNA, and *f* sheared MDCC-RP-1 DNA. o— — — o ^{14}C-labelled cellular DNA, o——— o MDV DNA sequences detected by filter hybridization with MDV ^{3}H-cRNA. The bottom of the gradients is to the left

gradient centrifugation (10). Ten μg DNA were loaded per gradient, and pseudorabiesvirus DNA was run in parallel as a 54 S sedimentation marker (4). *In vivo* ^{14}C-thymidine labelled cellular DNA was used to determine its sedimentation properties. The results shown in Fig. 2 are representative for both MDCC-MSB-1 and MDCC-HP-2 DNA, since their hybridization profiles were nearly identical. After velocity sedimentation some viral DNA was found at the 55 S position of free MDV DNA (Fig. 2, Fraction 18) followed by a slight viral DNA peak at about 65—70 S, which coincided with the size position of the bulk of the cellular

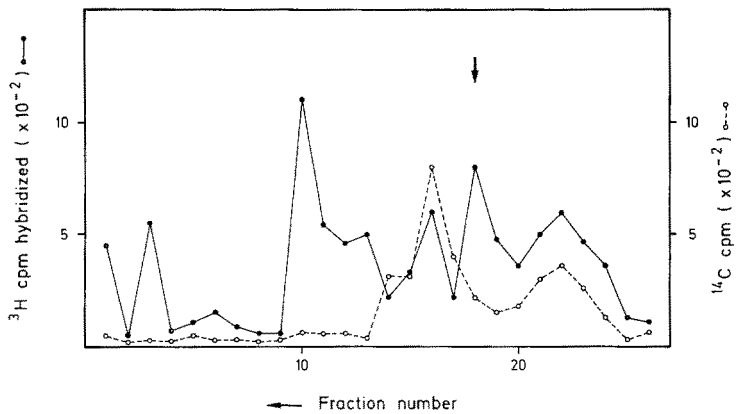

Fig. 2. Glycerol gradient centrifugation of free MDV DNA from MDCC-MSB-1 and MDCC-HP-2 cells pre-fractionated in CsCl-density gradients. Sedimentation was performed in 10—30 per cent glycerol gradients containing 1 M NaCl, 0.02 M Tris-HCl (pH 8.0), 0.001 M EDTA in a Beckman SW 27 rotor at 25,000 rpm/min and 20° C for 220 minutes. The arrow indicates the position of the 54S marker DNA. o— — — o ^{14}C-labelled cellular DNA, •———• MDV DNA sequences (hybridization with MDV ^{3}H-cRNA). Sedimentation was from right to left

DNA. Additionally, viral DNA sequences were also present in the fractions consisting of slow sedimenting cellular material (Fig. 2, Fractions 21—23), which might represent some breakdown products during the DNA preparation. A prominent viral DNA peak was reproducibly detected in the 100 S region (Fig. 2, Fraction 10), representing about 30 per cent of the free MDV DNA in both cell lines. This is expected for the sedimentation of covalently closed circular DNA molecules (10). Occasionally, an additional form of fast sedimenting viral DNA was observed, corresponding to about 140—150 S (Fig. 2, Fraction 3), which might be accounted for as catenated circular DNA dimers, as shown recently for EBV plasmid DNA (2, 5).

To confirm the presence of plasmid MDV DNA in MDCC-MSB-1 and MDCC-HP-2 cells, the 100 S DNA material of several glycerol gradients was pooled, and centrifuged to equilibrium in ethidiumbromide — CsCl gradients (10). As density markers supercoiled (I) and open circular DNA (II) of the human papovavirus BK was run in parallel. As shown in Fig. 3, about 35 per cent of the 100 S MDV DNA banded near the density of covalently closed supercoiled DNA (1.60 g/ml). This confirms the occurrence of MDV plasmid DNA in the MDCC-MSB-1 and MDCC-HP-2 cells. The remainder of the viral DNA was found at the density of

open circular DNA, which might be due to mechanical breakdown of the supercoiled DNA molecules. Finally, to provide further evidence for episomal MDV DNA in both cell lines, we tried to visualize circular DNA by electron microscopy. After concentrating the appropiate gradient fractions, single DNA molecules could be observed, which might be originally of circular structure. However, we failed to demonstrate unequivocally circular MDV DNA in these cell lines. This might be due to the low yield of the 100 S viral DNA material, which is not sufficient for a successful demonstration by electron microscopy. One might speculate that

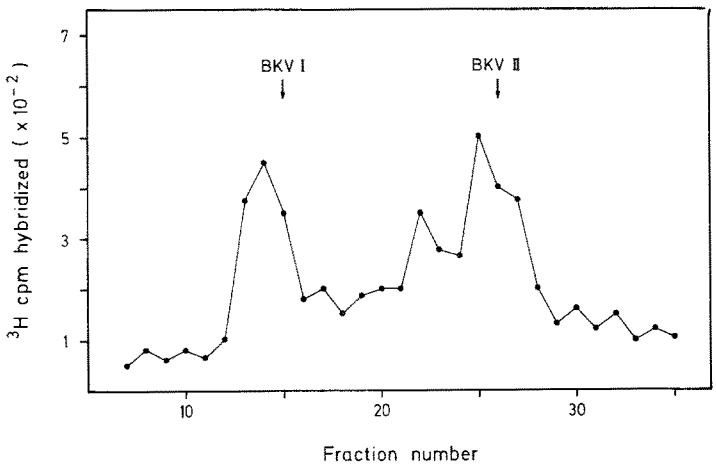

Fig. 3. Ethidiumbromide—CsCl—density gradient centrifugation of 100S MDV DNA from MDCC-MSB-1 and MDCC-HP-2 cells. The 100S DNA material was mixed with BKV-^{14}C-DNA, and centrifuged in an ethidiumbromide (350 μg/ml) containing CsCl gradient (final density 1.57 g/ml) for 50 hours at 35,000 rpm/min and 20° C in a 50 Ti rotor. After removing the dye the fractions were hybridized with MDV ^{3}H-cRNA. The bottom of the gradient is to the left

the ratio of integrated to episomal DNA in the cell lines is shifted to integrated viral DNA.

Circular plasmid DNA molecules have been demonstrated in a number of EBV-transformed cells (for review see 2), as well as in herpesvirus saimiri-transformed cells (17). The state of MDV DNA in virus-transformed cells has not been clearly defined. Previously, it was shown in the nonproducer MKT-1 cell line that viral DNA occured mainly in a circular plasmid form (16). These findings are corroborated by our results. There, we could demonstrate in another nonproducer cell line (MDCC-HP-2) as well as in a producer cell line (MDCC-MSB-1) the presence of MDV DNA circular molecules in addition to integrated viral DNA. However, in the third cell line investigated, MDCC-RP-1, viral DNA appears to be mainly associated with the cellular genome. The failure to detect circular DNA in those cells might be due to a low yield of viral DNA, which is under the detection level of the available techniques. Alternatively, it remains to be elucidated whether the integration of viral DNA into cellular DNA, found not only in MDV-transformed, but also found in lytically infected cells (7), or the formation of circular viral DNA is mandatory for a herpesvirus-induced cell transformation.

Acknowledgments

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