Mode of Integration of Epstein–Barr Virus Genome into Host DNA in Burkitt Lymphoma Cells

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SUMMARY

The EBV DNA integrated into the host genome from cells of an African Burkitt lymphoma biopsy has been compared to the corresponding fraction of EBV DNA from the cell line SU-AmB-2 which is of American Burkitt lymphoma origin. It is shown that while, in the case of the African biopsy, large pieces of EBV DNA sequences are integrated into the host DNA, much smaller pieces of virus DNA are integrated into the DNA of the SU-AmB-2 cells. The possibility that this difference might be related to the fact that EBV is rarely associated with Burkitt lymphomas outside East Africa is discussed.

The genome of the Epstein–Barr virus, (EBV), has been detected in neoplastic cells of unusual human tumours, the Burkitt lymphoma (BL) and the low differentiated nasopharyngeal carcinoma (NPC; Kaschka-Dierich et al. 1976). While it was believed that EBV is associated only with the Burkitt lymphomas of a very limited region of the world (East Africa), recent reports suggest that EBV is also associated with very few Burkitt lymphomas which have been found in other regions and which appear to be of the African type (Andersson et al. 1976; Epstein et al. 1976; Gravell et al. 1976). Recently we have reported the presence of EBV DNA in cells of a cell line which was established from an American BL (Koliais et al. 1978). We have detected both free circular EBV DNA of genome size and integrated sequences similar to the EBV DNA found in the cells of African BL biopsies and cell lines of BL origin. Because it is possible to isolate free circular EBV DNA, in relatively pure form, it has been studied in some detail (Lindahl et al. 1976). On the other hand very little is known about how the virus DNA is integrated in the host genome. It is not known whether the whole or part of the EBV genome is integrated and whether there are in the host DNA one or more integration sites. Because of its large size (10^8 daltons) there is no known restriction endonuclease which could cleave EBV DNA at only one site which would provide the answer to these questions by analogy with the SV 40 DNA which is cleaved by the restriction endonuclease EcoRI into two pieces (Morrow & Berg, 1972; Mulder & Delius, 1972).

In this communication the integrated EBV DNA which is contained in cells of BL origin is described. Advantage has been taken of the fact that EBV DNA has a higher buoyant density in CsCl than the host (human) DNA. DNA molecules which contain both virus and host sequences should have an intermediate density, the precise value depending on the proportion of the virus and host sequences. If care is taken to isolate large molecules of DNA, then the proportion of virus sequences in these molecules should be small and they should have a density near to that of host DNA. If, on the other hand, the size of these molecules is reduced, the proportion of virus sequences is increased in some pieces and the measured density, after the size reduction of the DNA, should be closer to that of virus DNA. Whether this actually happens depends on the size of the integrated sequences.

The source and various details of the BL cells (SU-AmB-2 cell line and Burkitt lymphoma biopsy cells) are described elsewhere (Koliais et al. 1978). The procedures used for the isolation of high mol. wt. DNA from the cells, fractionation of the DNA by CsCl density gradient centrifugation and the characterization of the virus DNA by hybridization with EBV cRNA are described by Lindahl et al. (1976) and by Kaschka-Dierich et al. (1976).
High mol. wt. DNA from the cell line SU-AmB-2 and the African BL biopsy was fractionated by neutral CsCl density gradient centrifugation in which a proper radioactive marker-DNA was included ($^3$H-DNA from *Klebsiella pneumoniae*, density 1.717 g/ml). The EBV DNA was detected by nucleic acid hybridization, a peak of cellular DNA was present at $\rho = 1.700$ g/ml, whereas the virus DNA in both cases was detected as a single peak at $\rho = 1.715$ g/ml (Fig. 1). The fractions with lower density than the EBV DNA, but a little higher than the host DNA (densities between 1.700 and 1.708 g/ml) were pooled. Half the amount of each was re-banded in the same type of gradient; the other half was also re-banded, but the size of the DNA was first reduced by repeated rapid passage through a no. 25 hypodermic syringe needle. This treatment should have reduced the size of the DNA to $8 \times 10^6$ daltons (Adams et al. 1973). As shown in Fig. 2, reduction of the size of the DNA from the African biopsy causes a shift of the EBV sequences to higher densities, in agreement with previously reported results by Adams et al. (1973), when they sheared corresponding fractions of EBV DNA from a cell line of African Burkitt lymphoma origin, and by Kaschka-Dierich et al. (1976), when they did similar experiments with another African BL biopsy. In the case of the DNA from the cell line SU-AmB-2, the same reduction in size of the DNA has no apparent effect on the density of the DNA.

In order to ensure that the reduction in size of the host DNA in the case of the SU-AmB-2 cells does not cause any shift of the apparent density of EBV DNA, the shearing experiment was repeated using the appropriate fractions of one parallel gradient to that shown in Fig. 2(b). (In this case one more CsCl gradient is included, so that the reduction in size is applied
Fig. 2. Re-banding of DNA from African BL biopsy cells and SU-AmB-2 cells in a neutral CsCl gradient. The fractions of density 1.700 to 1.708 g/ml were pooled and after the addition of new marker DNA each was divided into two parts. One part of each was sheared by six rapid passages through a No. 25 hypodermic syringe needle. The four DNA solutions were individually re-banded in CsCl under the same experimental conditions as in Fig. 1. (a), (b) Unsheared African Burkitt lymphoma biopsy cells and SU-AmB-2 cells, respectively; (c), (d) sheared African Burkitt lymphoma biopsy cells and SU-AmB-2 respectively. •—•, \textit{K. pneumoniae} \textsuperscript{3}H-DNA; ■—■, EBV DNA sequences. The arrows show the position of the cellular DNA.

to a more homogeneous population of molecules as far as the density is concerned.) Even in this case it was impossible to detect any change of the apparent density of the EBV DNA sequences (results not shown). These experiments show that in the case of the African BL, the EBV DNA sequences which are integrated in the host genome are of very large size. It is not possible to say whether the whole EBV genome is represented in the integrated fraction of the EBV genome. In the case of the DNA from the SU-AmB-2 cell line these
data show that the shearing of the total DNA has no effect on the density of the EBV DNA and therefore the integrated DNA is of very small size.

Since the biopsy and the SU-AmB-2 cells contain the same amount of EBV DNA (59 and 58 EBV genome equivalents per cell respectively; Koliais et al. 1978) and because it is evident from Fig. 1 and 2 that about the same proportion of this DNA is integrated into host DNA, it can be concluded that in the cells of the African BL biopsy, where large pieces of EBV DNA are integrated, few sites of integration exist, whereas in the cells of the American BL line, where small pieces of EBV DNA are integrated, a larger number of integration sites exists.

The difference reported here between the integrated DNA of cells of African and American Burkitt lymphomas is the only one found so far. It is possible that BL is extremely rare outside East Africa because EBV cannot latently infect the appropriate cells with high efficiency. This could be attributed to a less effective integration of the virus DNA in the host genome which is possibly reflected in the small size of the integrated DNA and/or the high number of integration sites in the SU-AmB-2 cells.

This hypothesis is in agreement with the findings of Battula & Temin (1977) who showed that integration of spleen necrosis virus DNA at single or multiple sites results in acute or chronic infection, respectively, of the chicken cells. However, this will not be clarified before more EBV positive lymphoma cell lines from non-African patients are established.

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REFERENCES


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