Circular and Circular-Linear DNA Molecules of Herpes Simplex Virus

(Accepted 9 June 1977)

SUMMARY

Circular and circular-linear DNA molecules of herpes simplex virus were isolated from infected cells. Two types of circular-linear DNA molecules are described, one with a circular component of about 8 µm and a linear component of 45 µm and the other with a circular component of 45 µm and a linear component of 8 µm. The circular DNA molecules were either the size of linear DNA molecules or were shorter and corresponded to the length of the L (long) component of linear virus DNA.

Linear double-stranded DNA molecules with a mol. wt. of \(100 \times 10^6\) can be found in virions and as uncoated virus genomes in the nuclei of cells lytically infected with a herpesvirus. Electron microscopy of herpesvirus DNA, separated from cellular DNA by centrifugation in CsCl density gradients, revealed mostly linear double-stranded DNA molecules although virus genomes having different conformations were also seen. Circular DNA molecules were described in pseudorabies virus infected cells (Jean & Ben-Porat, 1976) and in herpes simplex virus infected cells (Friedmann, Shlomai & Becker, 1977). Circular Epstein–Barr virus DNA molecules were isolated from Raji cells (Lindahl et al. 1976). The present study extends our previous findings on herpes simplex virus (HSV) DNA (Friedmann et al. 1977) and describes virus DNA molecules with a complete or partial circular conformation.

The HF strain of herpes simplex virus type I was used to infect BSC-1 cells. The virus DNA was isolated in CsCl density gradients and studied by electron microscopy as described by Friedmann et al. (1977). The following circular forms of virus DNA were observed: (a) Circular DNA molecules with a contour length (48 to 52 µm), similar to that of linear DNA genomes (52 ± 3 µm; Friedmann et al. 1977). A circular DNA molecule with a contour length of 48.3 µm is shown in Fig. 1. (b) Circular DNA molecules with contour lengths of 43.2, 45.0 and 45.1 µm, that are shorter than linear DNA genomes. (c) A DNA molecule with a large circular component (contour length of 45.1 µm) attached to a linear component of about 8 µm is shown in Fig. 2(a). (d) A DNA molecule with a small circular component (contour length of 8.8 µm) attached to a linear component longer than 40 µm is shown in Fig. 2(b). (Only a portion of the linear component is shown in Fig. 2b.) The junction between the circular and linear portions of the DNA molecule is shown in Fig. 2(c, d).

The fully circular HSV DNA molecules resemble pseudorabies (Jean & Ben-Porat, 1976) and Epstein–Barr virus circular genomes (Lindahl et al. 1976). Such circular DNA molecules could result from an interaction between the molecular ends of a linear DNA molecule. However, the presence of HSV DNA molecules that are partly circular and partly linear is more difficult to explain. It is proposed that the large circular portion of the molecule which measures 45 µm (Fig. 2a) may correspond to the L (long) component of HSV DNA while the linear portion of the DNA molecule (about 8 µm) may correspond to the S (short) component (Sheldrick & Berthelot, 1974; Wadsworth, Jacob & Roizman, 1975; Clements, Cortini & Wilkie, 1976). There are also DNA molecules consisting of a circular component
measuring 8.8 μm that may correspond to the S component and a large linear portion that may correspond to the L component.

The existence of HSV DNA molecules that are partly circular and partly linear in the nuclei of infected cells, suggests that they are formed by a unique mechanism. There is only a small number of such molecules in lytically infected cells since the virus DNA population consists mainly of linear DNA molecules. This indicates that the circular virus DNA molecules are either unstable or are formed by a mechanism that rarely operates. We suggest that the circular and the circular-linear DNA molecules may be formed by a mechanism of intra-
Fig. 2. Circular-linear HSV DNA molecules. (a) The contour length of the circular component of the DNA molecule is 45.1 µm. The arrow indicates the junction between the circular and linear portions of the DNA molecules. The linear portion of the DNA molecule is on the right hand side of the arrow. (b) The contour length of the circular component of the DNA molecule is 8.8 µm. The arrow indicates the junction between the circular and linear portions of the DNA molecule. The linear portion of the DNA molecule is on the left hand side of the arrow (only part is shown). (c) Enlargement of the junction region between the circular and the linear components of the DNA molecule in (a). (d) Enlargement of the junction region between the circular and linear components of the DNA molecule in (b).
molecular recombination (Skare & Summers, 1977) that gives rise to 8-shaped virus DNA molecules (Y. Becker, to be published). These DNA molecules are formed as a result of recombination of the two molecular ends of the linear DNA genome with the repetitious sequences situated between the L and S components of the molecule (Sheldrick & Berthelot, 1974). The circular DNA molecules are thought to be cleaved by a specific restriction-type enzyme at two positions in the X-shaped junction of the 8-shaped molecule. The circular-linear virus DNA molecules most probably arise due to cleavage of the 8-shaped molecule at one site only. In such DNA molecules either the L or the S component can have a circular form. The electron micrographs of the X-shaped junction in the circular-linear DNA molecules shown in Fig. 2(c, d) indicate that this region arises as a result of intramolecular recombinations.

We wish to acknowledge the help of Mrs Ester Kessler in the electron microscopy. The study was supported by contract NO1 CP 33310 from the Special Cancer Virus Program, NIH, Bethesda.

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(Received 18 March 1977)