JOURNAL OF VIROLOGY, Apr. 1977, p. 232-234

Vol. 22, No. 1
Printed in U.S.A.

Inverted Repetition in the Chromosome of Pseudorabies Virus

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Received for publication 5 October 1976

An electron microscope examination of pseudorabies virus DNA single strands after self-annealing shows a loop of single-stranded DNA at one end of the molecule contiguous to a double-strand region. The molecule then terminates in a further single-stranded region that does not form a loop. It is suggested that the DNA contains a sequence of 13.3×10^6 daltons at one end, which is repeated internally with opposite polarity. The segment of the genome separating the repeats has a double-strand molecular weight of 5.4×10^6. The whole native DNA has a molecular weight of 90×10^6 to 95×10^6.

The DNA of herpes simplex virus type 1 and that of type 2 have been shown to consist of two unequal regions, each bounded by inverted redundant sequences. This has been demonstrated by electron microscopy of self-annealed single strands of the herpes genome (5, 8). Such studies show a dumbbell arrangement, with unequal circles of single-stranded DNA at either end of a region of double-stranded DNA.

An analysis of the fragments produced by restriction endonuclease treatment of the native DNA of the viruses has been found to be consistent with the interpretation of the electron micrographs (2, 3).

The DNA of pseudorabies virus (pig herpesvirus 1) is similar in nucleotide doublet frequency to that of herpes simplex virus when subjected to nearest-neighbor analysis (7), and

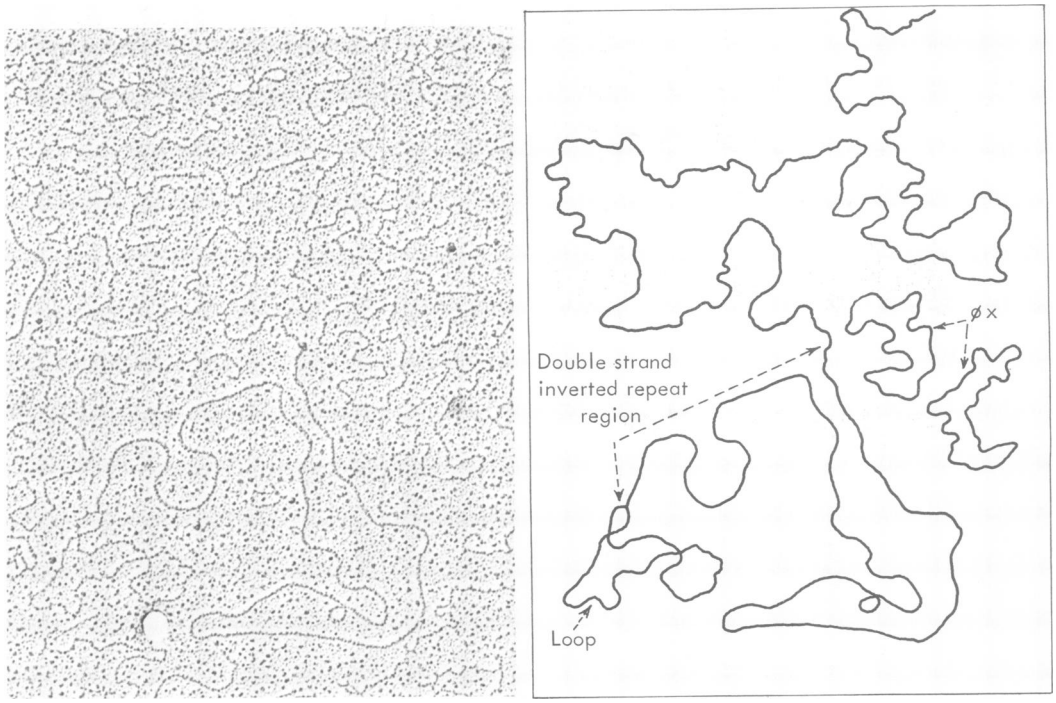

FIG. 1. *Electron micrograph of self-annealed pseudorabies DNA, showing the duplex repeat region and the single-stranded loop. ϕX174 circular DNA served as a marker. A tracing of the molecules is also shown.*

it has a molecular weight of 90×10^6 to 95×10^6 when examined by analytical ultracentrifugation (R. Eason and W. S. Stevely, unpublished data), which is similar to the figures reported for herpes simplex virus (5, 8). When single-stranded DNA from the virus was examined in an electron microscope after self-annealing, I found that there is a single inverted sequence that is represented at one end of the molecule as well as internally. The length of the repeated sequence and that of the DNA between the repeats are not identical to that of herpes simplex virus.

DNA was isolated from purified virions. These were obtained as previously described (6). The pellet of virus particles was suspended in Pronase and sodium dodecyl sulfate and incubated at 37°C for 16 h. Caesium chloride was added to the digest to give a refractive index of 1.3990. Precipitated sodium dodecyl sulfate was removed by brief centrifugation. Centrifugation to equilibrium gave a sharp band of virus DNA, which was collected, heated to 100°C for 20 min, made to 40% in formamide and to 2 M in urea, and then allowed to self-anneal for 3 h at 25°C. The DNA concentration was about 1 μg of DNA per ml.

ϕX174 DNA, in the form of single-stranded circles, was added, and the mixed DNA was spread for examination in an electron microscope, as described by Wellauer and David (9), whose method is a modification of that of Robberson et al. (4). The DNA in formamide and urea with cytochrome *c* is picked from a water hypophase, using a collodion-covered grid, and shadowed with platinum-palladium. Grids were examined in an AEI EM6 microscope at ×20,000 magnification. Plates were projected onto the screen of a Nikon comparator at ×10 magnification, and the contour of the molecules was traced for subsequent measurement with a map measure. Lengths were expressed as multiples of that of ϕX DNA circles from the same grid.

Figure 1 shows a micrograph of a section of a pseudorabies virus DNA single strand that has been self-annealed. There is a single-stranded loop contiguous to a double-stranded region, which terminates in a single-stranded tail. Molecules containing double loops were never seen.

Figure 2 is a histogram of the measured lengths of the single-stranded loops and of the double-stranded region indicative of the repeat. Not all of the duplex regions could be measured due to the proximity to the grid edges and the tangling of the DNA.

An analysis of the results gave the loop as

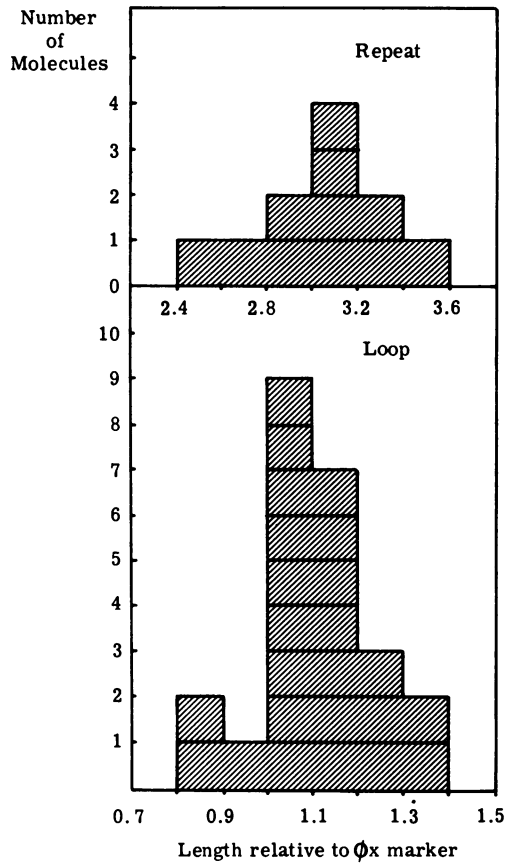

FIG. 2. *Histogram of the lengths of the single-stranded loop and the duplex repeat region of self-annealed pseudorabies DNA. Lengths are plotted relative to ϕX174 marker DNA.*

1.13 ± 0.13 times the ϕX marker. After correcting for the effect of the high guanine plus cytosine content of the virus on the single-strand length (5), a value of 2.7×10^6 daltons for the single-strand loop is obtained, which therefore corresponds to a double-strand region of 5.4×10^6 daltons in the original virus DNA.

The double-strand region formed by self-annealing was 3.0 ± 0.3 times as long as the marker. According to Bujard (1), the linear density values for double- and single-stranded DNA are 2.15×10^6 and 1.65×10^6 daltons/μm, respectively. This suggests that the sequence that is repeated has a molecular weight of 13.3×10^6.

An analysis of intact native DNA in an electron microscope gave a molecular weight for the molecule of 92×10^6.

In their analysis of herpes simplex virus, Wadsworth et al. (8) found that the combined lengths of the two repeated regions at either

end of the molecule had a molecular weight of 9.9×10^6 and that the shorter loop seen in their micrographs corresponded to a double-strand molecular weight of 9.0×10^6.

That pseudorabies virus DNA has a single inverted repeat region as described here has also been shown by D. Powell, J. B. Clements, and N. M. Wilkie (personal communication) by electron microscopy and restriction enzyme analysis of the DNA.

I am grateful to A. M. Campbell for the gift of the ϕX174 DNA and also to D. Powell, J. B. Clements, and N. M. Wilkie for communicating their results prior to publication.

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