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NOTES

Denaturation Pattern of the Deoxyribonucleic Acid from Chicken Embryo Lethal Orphan Virus

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The denaturation pattern of chicken embryo lethal orphan virus deoxyribonucleic acid confirms that the sequences are unique. The molecular weight of the deoxyribonucleic acid was determined by length measurements to be 29×10^6.

An understanding of the physical and chemical properties of a deoxyribonucleic acid (DNA) is required to study and understand the replication or the functioning of that DNA. The physical mapping of a viral genome can be of considerable value in relating functions (genes) to particular areas of the chromosome; this has been particularly so for bacteriophage lambda (1). We have studied the physical and chemical properties of chicken embryo lethal orphan (CELO) virus DNA to establish a physical map of the chromosome and a better understanding of the replication, transcription, and oncogenic potential of this avian adenovirus.

We have reported (5) that the DNA of CELO virus is a linear duplex molecule with a molecular weight of approximately 30×10^6. We also presented evidence (7) that the DNA sequence was unique, rather than circularly permuted, and contained regions which were rich in adenine-thymine base pairs. As a further characterization of the physical properties of the CELO virus chromosome, we present a denaturation map of the DNA and a more accurate measurement of the molecular weight.

CELO virus DNA was prepared from virus which was grown in embryonated eggs and purified as described previously (5).

To determine the molecular weight, the CELO virus DNA was mixed with a preparation of ϕX174 RFII DNA molecules (a gift from L. Smith, California Institute of Technology) and spread by the aqueous technique of Davis et al. (2). The grids were stained with uranyl acetate (2) and photographed in a Siemens electron microscope. The negatives were projected onto paper and traced, and the lengths of three ϕX174 RFII DNA molecules appearing on the same negative with each CELO virus DNA molecule were measured. The ratio of the length of CELO virus DNA to ϕX174 RFII DNA was 8.52 ± 0.19 (mean $\pm$ 95% confidence limits for the mean). The molecular weight of the replicative form of ϕX174 DNA is 3.4×10^6 (6); thus, we calculated the molecular weight of CELO virus DNA $29.0 \pm 0.65 \times 10^6$, a calculation in close agreement with the molecular weight calculated from sedimentation studies (2).

Partial denaturation of CELO virus DNA was achieved by the alkaline method of Inman and Schnös (4). CELO virus DNA was exposed to *p*H 10.9 in the presence of 10% formaldehyde. Samples were removed at various times, neutralized (4), and examined in an electron microscope by the formamide technique of Davis et al. (2). Denatured regions were just begining to appear at 30 min and, of 58 molecules photographed from this sample, 23 (40%) had no denatured regions, 27 (47%) had one denatured region, and the remaining 8 (14%) had two denatured regions. The denatured regions were very small, rarely exceeding 1% of the molecular length, and were all located near one end of the molecules (Fig. 1a and 2). Of those molecules which had denatured regions, 83% had a denaturation located between 0.95 and 0.97 fractional length units from the left-hand end of the molecule (the molecules were oriented with the major denaturing region to the right to agree with the orientation of the denaturation map of human adenovirus type 2 DNA [3]).

DNA samples which were examined after 60 min at *p*H 10.9 had a number of denaturation regions on each molecule. A distinctive pattern

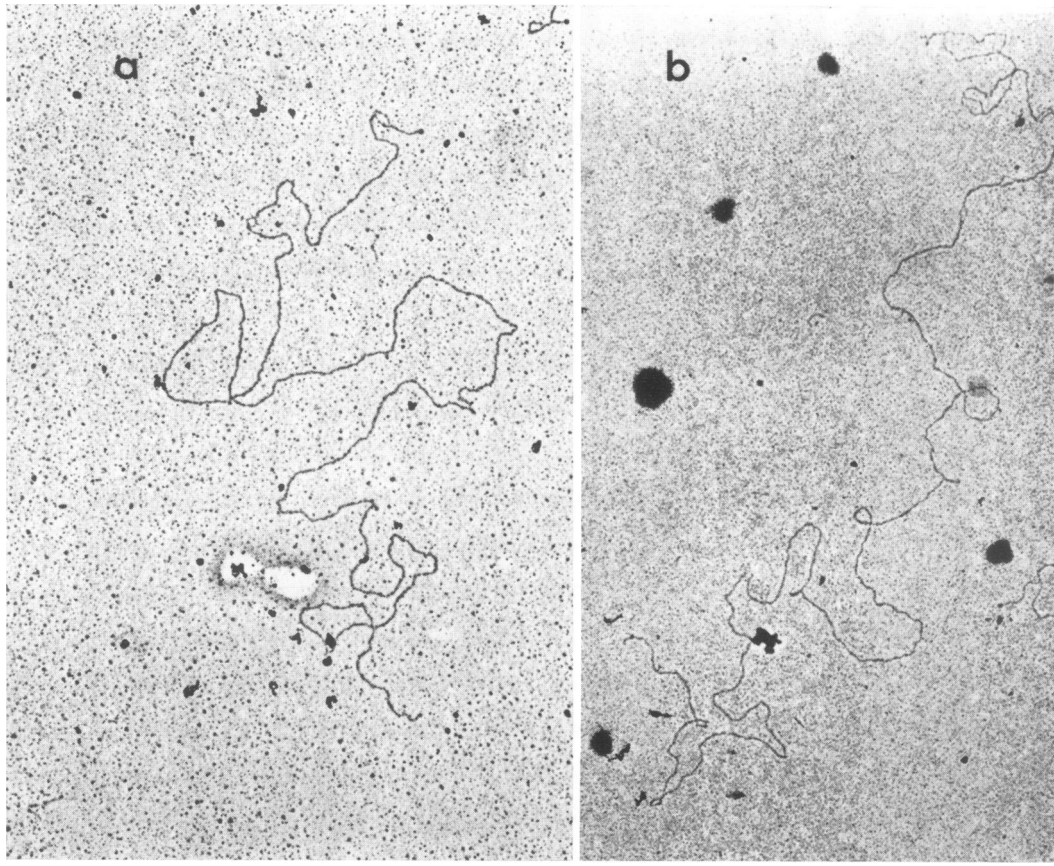

FIG. 1. *Chicken embryo lethal orphan virus DNA molecules which have been partially denatured at pH 10.9 in 10% formaldehyde for (a) 30 min and (b) 60 min.*

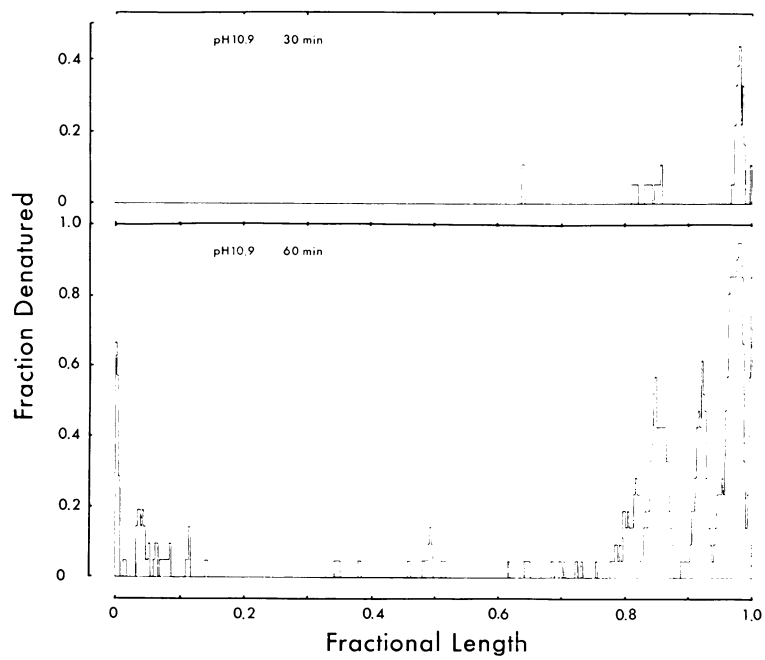

FIG. 2. *Frequency distribution of the denaturation sites in chicken embryo lethal orphan virus DNA resulting from pH 10.9 for 30 and 60 min. The fraction of molecules which were denatured was determined for 400 equal lengthened positions along the molecule and plotted versus the fractional length of the DNA molecule.*

occurred at one end of each molecule (Fig. 1b and 2) which made orientation of the molecules very simple.

The peaks on a histogram at 0.85, 0.92, 0.96 and 1.00 are extremely well defined with almost no overlap. This suggests that the adenine-thymine-rich regions are separated by relatively guanine/cytosine-rich regions. The overall impression from the 60 min sample is that the right-hand 25% of the molecule contains the majority of the adenine-thymine-rich regions.

DNA which had been exposed to *p*H 10.9 for 140 min was extensively denatured and extremely difficult to orientate. Those molecules which could be oriented had large denatured regions at both ends; the small denatured region at 0.50 fractional-length units in the 60 min sample (Fig. 2) was greatly increased and a new region at 0.36 had appeared. The regions from 0.39 to 0.48 and from 0.55 to 0.71, however, remained relatively free of denaturation. A number of molecules also had a short undenatured region at 0.87 which corresponds with one of the interpeak areas of the 30 min sample (Fig. 2).

These results confirm our earlier finding (5) that the DNA of CELO virus is not circularly permuted and does not have long terminal redundancies. It also provides a physical map of the chromosome which may be of value in other studies on the DNA.

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LITERATURE CITED

1. Davidson, N., and W. Szybalski. 1971. Physical and chemical characteristics of lambda DNA, p. 45–82. *In* A. D. Hershey (ed), The bacteriophage lambda. Cold Spring Harbor Press, Cold Spring Harbor, New York.
2. Davis, R. W., M. Simon, and N. Davidson. 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids, p. 413–428. *In* L. Grossman and K. Moldave (ed), Methods in enzymology, vol 21. Academic Press Inc., New York.
3. Doerfler, W., and A. K. Kleinschmidt. 1970. Denaturation pattern of the DNA of adenovirus type 2 as determined by electronmicroscopy. J. Mol. Biol. **50**:579–593.
4. Inman, R. B., and M. Schnös. 1970. Partial denaturation of thymine and 5-bromouracil-containing λ DNA in alkali. J. Mol. Biol. **49**:93–98.
5. Laver, W. G., H. B. Younghusband, and N. G. Wrigley. 1971. Purification and properties of chick embryo lethal orphan virus (an avian adenovirus). Virology **45**:598–614.
6. Sinsheimer, R. L. 1959. A single stranded deoxyribonucleic acid from bacteriophage $\phi X174$. J. Mol. Biol. **1**:43–53.
7. Younghusband, H. B., and A. J. D. Bellett. 1971. Mature form of the deoxyribonucleic acid from chick embryo lethal orphan virus. J. Virol. **8**:265–274.