CHARACTERIZATION OF MYCOPLASMATALES VIRUS DNA

Alan Liss and Jack Maniloff*

Departments of Microbiology and of
Radiation Biology and Biophysics
University of Rochester
Rochester, New York 14642

Received January 29,1973

SUMMARY

The DNA of the group L1 Mycoplasmatales virus, MVL51, was analyzed using alkaline sucrose velocity sedimentation, neutral and alkaline CsCl isopycnic sedimentation, and treatment of the DNA with nucleases. These treatments show that the viral chromosome is a covalently linked single-stranded DNA circle of molecular weight 2×10^6 daltons.

INTRODUCTION

Recently, three morphologically and serologically distinct types of viruses infecting members of the order Mycoplasmatales have been isolated (1,2,3,4). Two of these groups, group L1 and group L2, are known to be DNA viruses (3). This paper reports the first detailed characterization of the DNA of a Mycoplasmatales virus, the group L1 virus MVL51.

MATERIALS AND METHODS

Cells, virus and medium

The cell used as the virus indicator strain and to propagate the virus was Acholeplasma laidlawii BN1-Nal^r (1), to be referred to as JA1. The MVL51 virus was isolated from a rare spontaneous plaque on an uninfected JA1 lawn, as reported by Liss and Maniloff (5). In all cases, JA1 cells were cultivated in tryptose broth or on tryptose agar plates (1).

Labelling of MVL51 DNA and Marker DNA

In all gradient experiments, DNA from the filamentous Escherichia coli bacteriophage, fd, was used as a marker molecule. This virus was kindly supplied by Dr. D. A. Marvin (Yale Univ.). The fd was labelled with

*Visiting scientist, 1972-73, John Innes Institute, Colney Lane, Norwich NOR 70F, England.

^{3}H-thymidine (New England Nuclear, Boston, Mass.) as described by Wirtz and Hofschneider (6) and used within four days after preparation. ^{32}P labelled MVL51 was prepared by adding 2mCi of ^{32}P carrier-free orthophosphate (New England Nuclear, Boston, Mass.) to 10ml of infected JA1 cells growing in Eagle's minimal essential medium (7). After incubation in this medium for eight hours at 37°C, viruses were isolated and DNA extracted as previously described (5).

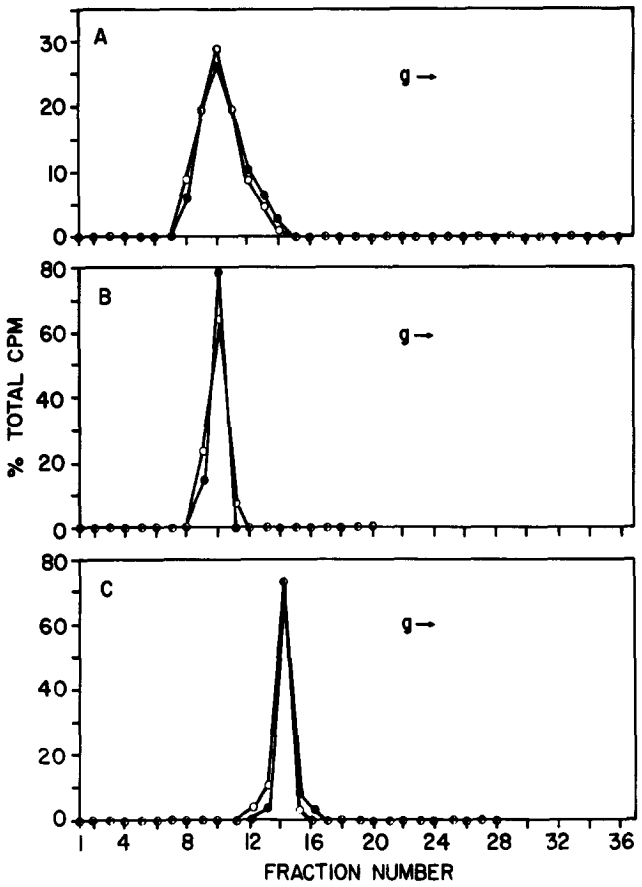
Centrifugation analyses

Alkaline sucrose gradient velocity sedimentation followed the protocol of Das et al (8). Viral DNA was layered on 5.0 ml of a 5-20% (w/v) sucrose gradient, adjusted to pH 12.0 with NaOH. The samples were centrifugated in the SW50.1 rotor for two hours at 28,000 rpm at 20°C in a Beckman L3-50 centrifuge. After centrifugation, drops were collected from the bottom of the tubes and precipitated with an equal volume of cold 10% (w/v) trichloroacetic acid. The precipitate was collected on HAWP Millipore filters, washed with distilled H_2O and 95% ethanol and then placed in 9 ml of Aquasol (New England Nuclear, Boston, Mass.) plus 1 ml of H_2O. Radioactivity ^{32}P or ^{3}H was counted in a Beckman LS-230 liquid scintillation counter.

CsCl isopycnic sedimentation followed the protocol of Tseng and Marvin (9). Neutral equilibrium gradients contained 2.65 g of CsCl and 2.0 g of sample in 0.05M tris(hydroxymethyl)aminomethane (Tris)- 0.003M ethylenediaminetetraacetate (EDTA), adjusted to pH 8.1 with HCl. Alkaline equilibrium gradients contained 4.0 g of CsCl and 2.9 g of sample in 0.05M Na_3PO_4-0.003M EDTA, adjusted to pH 12.5 with 1N NaOH. The gradients were run in non-wetting polyallomer tubes for 48 hrs at 17°C in a SW50.1 rotor at 27,000 rpm. Fractions were collected as described above.

Enzymic treatment of MVL51 DNA

MVL51 DNA was treated with Escherichia coli exonuclease I, which hydrolyzes single-stranded DNA. This enzyme was a gift from Dr. Robert Swift (Univ. of Rochester). The reaction mixture for the enzyme involved the



(1A) Alkaline sucrose velocity sedimentation. DNA samples were layered onto a 5-20% sucrose gradient (pH 12.0) and sedimented for 2 hrs at 28,000 rpm at 20°C in a SW50.1 Beckman rotor.

(1B) Neutral CsCl equilibrium sedimentation. DNA was mixed with a solution of CsCl, pH 8.1 at a density of about 1.71 g/cm^3. Sedimentation was for 48 hrs at 27,000 rpm at 17° C in a SW50.1 Beckman rotor.

(1C) Alkaline CsCl equilibrium sedimentation. DNA was mixed with a solution of CsCl, pH 12.5 at a density about 1.76 g/cm^3. Sedimentation was for the time and conditions mentioned for (1B).

In all cases, (O) represents ^{32}P and (●) represents ^{3}H counts. Each graph shows the percent of the total TCA precipitable material in each fraction. The variation in number of fractions in these figures arises because each figure represents a different type of centrifugation experiment.

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addition of 2 μg/ml of MVL51 DNA to an equal volume of exonuclease in a mixture of 0.2% bovine serum albumin, 0.005M $MgSO_4$, 0.05M glycine at pH 9.0. The 30 min incubation was carried out at 37° C. The action of this enzyme on MVL51 DNA was monitored as decrease in DNA infectivity in the transfection

system described by Liss and Maniloff (5). The activity of the exonucleases under these conditions was confirmed by Dr. Swift (unpublished results).

RESULTS AND DISCUSSION

Gradient analysis showed that, by alkaline sucrose velocity sedimentation (Fig. 1A), neutral CsCl isopycnic sedimentation (Fig. 1B), and alkaline CsCl isopycnic sedimentation (Fig. 1C), both MVL51 DNA and fd DNA had identical sedimentation properties. This was not a result of an interaction between the two DNAs, because, in parallel samples (data not shown), each of the DNA species was sedimented by itself and gave the same result as was observed for the mixture of the two DNAs.

The single MVL51 DNA peak, co-sedimenting with fd DNA, in the neutral and alkaline CsCl isopycnic experiments indicates that MVL51 DNA is a single-stranded circular molecule (10). The co-sedimentation in the velocity experiment shows that both DNAs have similar molecular weights, 2×10^6 daltons (10).

The circularity of the MVL51 chromosome was confirmed by nuclease treatments. Deoxyribonuclease I (an endonuclease) attacks the MVL51 DNA, measured as a loss in DNA infectivity (see also reference 5). In contrast, exonuclease I (which is specific for single-stranded DNA) has no effect on MVL51 DNA; after incubation with the nuclease 85-100% of the initial infectivity remained. Parenthetically, exonuclease III (specific for double-stranded DNA) had no effect on the MVL51 DNA.

In conclusion it should be noted that MVL51 and similar isolates are the only known DNA-containing bullet-shaped viruses (11). It is interesting that their chromosome structure (single-stranded circles, 2×10^6 daltons) is similar to that of the filamentous bacteriophages (12) and the icosahedral bacteriophage øX174 (13) and has the same size and topology of animal parvoviruses (14). This may indicate some general biological limit to the minimum size possible for such viral chromosomes.

ACKNOWLEDGEMENTS

We wish to thank Dr. J. R. Christensen and Dr. F. E. Young for many help-

ful discussions and Mr. David Gerling for his excellent technical assistance. This investigation was supported in part by grant AI 10605 from the United States Public Health Service, National Institute of Allergy and Infectious Diseases. AL is a USPHS predoctoral trainee (grant GM 00592) and JM is the recipient of a USPHS Research Career Development Award (grant AI 17480).

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