Integration and Lysogeny by an Enveloped Mycoplasma Virus

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SUMMARY

The 11.8 kilobase pair (7.8 x 10^6 mol. wt.) genome of mycoplasma virus L2 in lysogenic Acholeplasma laidlawii cells was examined. For this study, DNAs were analysed by agarose gel electrophoresis and viral DNA sequences identified by DNA–DNA hybridization. L2 DNA was found to be integrated into the lysogenic host cell chromosome at a unique site in both viral and cellular DNA. The viral DNA site was roughly mapped and the approximate time of integration during the L2 non-cytocidal infectious cycle was determined.

Mycoplasma virus L2 is an enveloped virus containing circular superhelical double-stranded DNA of 11.8 kilobase pairs (7.8 x 10^6 mol. wt.) (Lombardi & Cole, 1979; Nowak & Maniloff, 1979). L2 infection of Acholeplasma laidlawii is non-lytic and leads to the establishment of a lysogenic state (Putzrath & Maniloff, 1978). Clones of lysogenic cells were found to be resistant to superinfection by homologous virus, but able to be infected by heterologous virus. These cells had the potential to produce virus and transmitted this potential as a stable heritable trait. Mitomycin C and u.v. light induced an increase in infectious centres in cultures of lysogens. Therefore, in the studies reported here, such cells were examined by DNA–DNA hybridization to determine the state of the L2 viral genome they carry.

This study used A. laidlawii strain K2 host cells, mycoplasma virus L2 and tryptose growth medium, as described previously (Haberer et al., 1979; Putzrath & Maniloff, 1977). Cells were assayed as colony-forming units (c.f.u.) and viruses as plaque-forming units (p.f.u.).

To obtain virus, 200 ml overnight cultures of A. laidlawii strain K2 were infected with L2 (m.o.i. 1 to 10) and diluted to 1 litre with fresh tryptose broth. After overnight incubation at 37 °C, cells were removed by centrifugation (7 min at 9000 rev/min at 4 °C in a Beckman JA-14 rotor), and the virus pelleted (30 min at 25000 rev/min at 10 °C in a Beckman SW27 rotor). The virus was resuspended in 5 ml TES buffer (0.01 M-Tris-HCl pH 7.8, 0.1 M-NaCl, 0.001 M-EDTA) and purified by sedimentation (3 h at 25000 rev/min at 10 °C in a Beckman SW27 rotor) in a linear 15 to 30% (w/v) sucrose gradient. Fractions containing L2 were pooled and concentrated by centrifugation. After resuspension in 0.5 ml TES, the virus titre was 10^13 to 10^14 p.f.u./ml. Virus DNA was obtained by extracting twice with equal volumes of phenol saturated with TES. The aqueous layer was made 0.2 M in sodium acetate and DNA precipitated by addition of 2 vol. 95% ethanol. After overnight storage in the cold, DNA was harvested by centrifugation (1 h at 30000 rev/min at 0 °C in a Beckman SW50.1 rotor) and resuspended in 0.5 ml TES. DNA concentrations were typically about 1 mg/ml.

Restriction endonucleases were obtained from Bethesda Research Laboratories and the digestion conditions were as recommended. The protocol for mapping cleavage sites in L2 DNA by use of agarose gel electrophoresis was described previously (Nowak & Maniloff, 1979).

Several restriction endonucleases were used to construct a physical map of the L2 genome (Fig. 1). The HindIII and Hpal sites confirm previously published results (Nowak & Maniloff, 1979). Although only one XbaI site was originally identified (Nowak & Maniloff, 1979), two close XbaI sites were resolved in these studies (Fig. 1). Using L2 virus grown on A. laidlawii strain JA1, the single BglII site was chosen as the map zero-point (Nowak & Maniloff, 1979).
Fig. 1. Restriction endonuclease cleavage map of the mycoplasma virus L2 genome. The restriction endonuclease recognition sequences were mapped as described previously (Nowak & Maniloff, 1979). The parentheses around 'BgII' denote that this site is modified and not cleaved in L2 virus grown on K2 cells (Dybvig et al., 1982). The site at which L2 integrates into the cell chromosome is somewhere within the L2 map region indicated by the heavy line.

However, in the studies reported here, L2 virus was grown on A. laidlawii strain K2 and, due to the DNA modification system of K2 cells, DNA from L2 virus grown on K2 cells contained no BgII recognition site (Dybvig et al., 1982).

For studies of integration of viral DNA, DNA from uninfected and lysogenic cells was analysed by agarose gel electrophoresis. Viral DNA sequences were identified by DNA–DNA hybridization using the Southern blotting technique (Southern, 1975). The hybridization probe was L2 viral DNA labelled by nick translation.

Virus DNA and cell DNA (from 4-5 ml cultures) were purified as described above, except that cell DNA was precipitated with 70% ethanol and centrifuged for 15 min at 20000 rev/min. Some samples were treated with a restriction endonuclease, as specified in the appropriate figure legend. The DNAs were analysed by gel electrophoresis using 0-75% agarose slab gels at 40 mA for 3-5 h (Nowak & Maniloff, 1979). Gels were stained with ethidium bromide and photographed using u.v. light illumination. DNA was transferred from the agarose gel to 0.45 μm Millipore nitrocellulose filters by the method of Southern (1975). For the hybridization probe, L2 DNA was labelled with $^{32}$PdCTP by nick translation (Rigby et al., 1977). $^{32}$P-labelled L2 DNA was hybridized to the filter-bound DNA in 4 × SSC (1 × SSC was 0.015 M- NaCl, 0.015 M-sodium citrate, pH 7.0) containing 0.5% SDS, at 68 °C for 16 h. Filters were washed twice in 2 × SSC containing 0.5% SDS, each time in 500 ml for 30 min at 55 °C, and once with 1 litre 2 × SSC at 55 °C for 30 min. The filters were examined by autoradiography using Kodak X-ray film (XR-1).

To obtain lysogenic clones, an A. laidlawii strain K2 culture was infected with L2 virus, incubated overnight, and maintained by daily transfer for 2 days. After plating, single colonies were picked. In agreement with previous reports (Putrzath & Maniloff, 1977, 1978), all these lysogenic clones were resistant to L2 infection, and cultures of different clones contained different levels of free L2. The independent lysogenic clones 10, 17 and 31 were selected for study.
Fig. 2. Analysis of DNAs from L2 virus, lysogenic cells, and uninfected cells. Untreated and restriction endonuclease-treated DNAs, analysed by agarose gel electrophoresis, were stained with ethidium bromide (lanes a to j), transferred to nitrocellulose filters, hybridized against $^{32}$P-labelled L2 DNA, and examined by autoradiography (lanes k to t), as described in the text. The following DNAs were studied: (a, k) L2, (b, l) L2 + XbaI, (c, m) lysogenic clone 31, (d, n) clone 31 + XbaI, (e, o) lysogenic clone 10, (f, p) clone 10 + XbaI, (g, q) lysogenic clone 17, (h, r) clone 17 + XbaI, (i, s) uninfected K2 + XbaI and (j, t) uninfected K2. The positions of relaxed open circular (OC), linear (LIN) and superhelical covalently closed circular (CCC) L2 DNA are indicated. The amount of material in (n) makes it difficult to resolve the two close bands, but these are easily seen in (p) and (r).

by the Southern blot technique, since they had the lowest levels of free L2: overnight cultures (about $10^9$ c.f.u./ml) of clone 10 contained about $1 \times 10^5$ p.f.u./ml; of clone 17, about $6 \times 10^5$ p.f.u./ml; and of clone 31, about $2 \times 10^6$ p.f.u./ml.

Uninfected *A. laidlawii* K2 DNA contained no L2 DNA sequences (Fig. 2s, t). All three lysogenic clones, derived from L2-infected K2 cells, had L2 DNA sequences in high molecular weight DNA (Fig. 2m, o, q). Virus DNA in the high molecular weight band was not due to trapping of free virus DNA by high molecular weight cell DNA, but represented L2 DNA integrated into the cell genome, as shown by restriction endonuclease digestion studies.
Digestion of lysogenic clone DNA with XbaI showed that these cells contained L2 viral DNA integrated into their genomes. XbaI cut L2 DNA into two pieces (Fig. 1), one small (so that it ran off the gel under the experimental conditions used) and the other large, almost of unit length. The XbaI digests of lysogenic cell DNA showed two bands containing L2 DNA (Fig. 2n, p, r); both bands had a molecular weight higher than linear L2 DNA alone. Hence, XbaI cut lysogenic cell DNA in such a way that two of the fragments contained both viral and cellular sequences. Therefore, L2 DNA must be integrated, with the viral integration site somewhere within the large XbaI fragment.

There are two other conclusions from these data. (i) The difference in intensities between the two bands in each XbaI digest (Fig. 2n, p, r) indicates that the viral integration site is relatively close to the viral XbaI sites. (ii) The fact that similar digestion data were obtained for three independent lysogens indicates that there is a unique integration site in both viral and cellular DNA. Possible secondary sites were not detected in these studies.

These conclusions have been confirmed using AvaII, HindIII and HpaI restriction endonucleases (data not shown). From the data, the site of integration on the L2 genome has been roughly mapped (Fig. 1).

The non-cytocidal L2 infectious cycle has been shown to involve a 90 to 120 min latent period followed by a 4 to 6 h rise period and, finally, establishment of lysogeny (Putzrath & Maniloff, 1977). To investigate the time of integration, cells were infected and samples removed at 2 h intervals. For each sample, the DNA was extracted, treated with XbaI and analysed by the Southern blot technique (data not shown). Four h post-infection was the earliest time at which free intracellular viral DNA could be detected, probably because the DNA isolation procedures favoured the extraction of high molecular weight DNA. At 4 h, XbaI fragments of both free virus and integrated viral DNA were observed, and no significant change was seen in the amounts of these two types of fragments up to 10 h. Hence, measurable L2 integration occurred at 2 to 4 h of infection and free viral DNA remained in the cytoplasm until at least 10 h of infection. Sedimentation velocity experiments of infected-cell lysates have confirmed the presence of free viral DNA in cells late in infection, although virus DNA synthesis ceases at about 5 to 6 h post-infection (Dybvig, 1981).

In summary, these studies have shown that A. laidlawii cells lysogenized by mycoplasma virus L2 have a single L2 genome integrated in the cell chromosome. A single integration site was found in both viral and cellular DNA; no secondary integration sites could be detected in these studies. This is the first demonstration of recombination in mycoplasmas.

Integration of L2 occurs during the rise period in the non-lytic, non-cytocidal infection cycle. Hence, in an infected culture, progeny virus release continues after integration. Regulation of L2 integration is expected to be different from that of bacteriophage λ (Weisberg et al., 1977), since the two viruses have different types of infectious cycle; phage λ produces a lytic productive infection, while L2 has a non-lytic, non-cytocidal productive infection.

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**REFERENCES**


Short communication


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