Persistent virus integration into the genome of its algal host, *Ectocarpus siliculosus* (Phaeophyceae)

Nicolas Delaroque, Ingo Maier, Rolf Knippers and Dieter G. Müller

Fakultät für Biologie, Universität Konstanz, D-78457 Konstanz, Germany

The brown alga *Ectocarpus siliculosus* frequently carries an endogenous virus, *E. siliculosus* virus (EsV-1), the genome of which is a circular, double-stranded DNA molecule of about 320 kbp. After infection, which occurs in the unicellular spores or gametes, the virus is present latently in all somatic cells of the host. Virus multiplication is restricted to cells of the reproductive organs. It has been an open question whether the latent viral DNA occurs as a free episome or becomes integrated into the host genome. PCR studies showed that viral DNA co-migrates with high molecular mass DNA in pulsed-field gel electrophoresis, which confirms that latent viral DNA is integrated into the host genome.

Marine filamentous brown algae of the order Ectocarpales frequently carry endogenous viruses with large double-stranded DNA genomes (Müller *et al.*, 1998). A well-studied example is *Ectocarpus siliculosus* virus-1 (EsV-1), the icosahedral capsid of which encloses a circular genome of about 320 kbp (Müller *et al.*, 1990; Lanka *et al.*, 1993). Like other brown-algal viruses, EsV-1 exclusively infects cell wall-free zoospores or gametes. Infected cells develop into mature thalli which can produce pathological symptoms in their sporangia or gametangia. These organs become densely packed with viral particles that are eventually released into the surrounding seawater (Fig. 1a). It is not unusual, however, for infected algae to appear normal and to produce viable spores (Fig. 1d) containing the viral genome (Fig. 1e, lane 4). In fact, genetic studies and PCR analyses have shown that the latent viral genome is transmitted vertically through meiosis as a Mendelian trait (Müller, 1991a; Bräutigam *et al.*, 1995).

These earlier reports could not distinguish between an episomal free state of the EsV-1 DNA and integration into the host genome. We report here the results of experiments that were designed to distinguish between these possibilities. We prepared high molecular mass DNA from zoospores and gametes of infected and uninfected algae by using a modification of the protocol of Liu & Whittier (1994). The following clonal isolates of *E. siliculosus* were used: strain PAr 10n (Fig. 1b), a healthy female gametophyte (Müller, 1979; Sengco *et al.*, 1996); NZVicZ14 (Fig. 1a, c), which is infected by EsV-1 and produces virions as well as zoospores carrying a latent viral genome (Müller *et al.*, 1996; Sengco *et al.*, 1996); and strain Nap R-B1 (Fig. 1d), which is a phenotypically normal male gametophyte that is latently infected by EsV-1 and does not produce virions (Sengco *et al.*, 1996). The cultures of PAr 10n and NZVicZ14 were axenic, Nap R-B1 was unialgal. EsV-1 DNA was prepared from virions released by strain NZVicZ14 as described by Lanka *et al.* (1993). Culture conditions were as described by Müller (1991a, b). Mature algae were stored in the dark at 2°C overnight. Mass release of reproductive cells within 20 min was induced by rapid transfer into a small volume of fresh culture medium combined with a temperature increase to 18°C and exposure to light.

The cells were collected by centrifugation at 1400 g for 15 min, washed and resuspended in culture medium and then gently mixed with an equal volume of low-melting-point agarose (Biozym) prepared in culture medium. Agarose plugs were treated twice with 2 mg/ml proteinase K (Boehringer Mannheim) in 10 mM Tris–HCl (pH 8.0), 50 mM EDTA and 1% SDS for 16–18 h at 50°C. The agarose plugs were washed three times for 1 h each in 500 mM EDTA at 50°C and finally stored at 4°C. This procedure yielded large amounts of DNA that was suitable for restriction nuclease digestion (not shown) and pulsed-field gel electrophoresis.

Ethidium bromide staining showed a strong band of DNA at the limit of size separation of the gel system (approx. 600 kbp), although a considerable fraction of DNA was smeared over a region corresponding to lower molecular mass. Another part of the stainable DNA remained in the gel slots, probably due to incomplete proteolysis (Fig. 2). Ethidium bromide staining did not reveal the presence of free viral DNA (Fig. 2, lanes 3 and 4), except in experiments where spores of NZVicZ14 were accompanied by simultaneously released virions (Fig. 2, lane 5).
In order to detect virus sequences in the DNA preparations shown in Fig. 2, we first performed Southern blotting experiments. However, the hybridization signals were very weak and unreliable (data not shown), except where viral DNA could also be identified by ethidium bromide staining as in lane 5 of Fig. 2.

As an alternative, we used a PCR approach. Lanes were excised from gels like that shown in Fig. 2 and then cut into 0.5 cm slices. DNA was extracted from individual slices with β-agarase I (New England Biolabs). Samples of 1–3 µl were analysed by PCR by using EsV-1-specific primers and protocols from Bräutigam et al. (1995).

In a control experiment with purified EsV-1 DNA (Fig. 3 a), we obtained positive PCR signals from slices containing intact viral DNA as well as from slices with smaller viral DNA fragments, which arise because EsV-1 DNA tends to break at one or more of its many single-stranded regions (Klein et al., 1994). A second control experiment was performed with PAr 10n algal DNA, which confirmed the absence of virus DNA as expected (Fig. 3b). In contrast, all slices containing DNA of infected Ectocarpus cells contained viral DNA sequences, regardless of whether the DNA had been prepared from spores of NZVicZ14 (Fig. 3c) or gametes of Nap R-B1 (Fig. 3d). The important point here is that positive virus signals were found not only at the positions where intact or fragmented viral DNA could be expected, but also at positions corresponding to high molecular mass host DNA (compare Fig. 3d with Fig. 3a).

Concatemers of viral DNA can be excluded, since virus replication does not occur in the unicellular gametes or spores of the hosts that we used for the isolation of DNA (Müller et al., 1990). All known examples of concatemer formation are linked to viral DNA replication (Mosig et al., 1995; Boehmer & Lehman, 1997).

In order to exclude the possibility that viral DNA was bound, trapped or mechanically retained by cellular DNA aggregates, the 600 kbp complexes were excised from a gel similar to that shown in Fig. 2 and again subjected to electrophoresis and PCR analysis (Zimmer & Verrinder Gibbins, 1997). The results (not shown) were similar to those shown in Fig. 3. Thus, our data indicate a covalent association of viral and cellular DNA. Our conclusion is that EsV-1 DNA occurs as an integrated provirus in latently infected cells. Recently, Lee et al. (1998) claimed, without presenting
EsV-1 integration

Fig. 2. Pulsed-field gel electrophoresis. DNA was isolated from EsV-1 virions (lane 1), PAr 10n (2), NZVicZ14 (3 and 5) and Nap R-B1 (4). The DNA (2–3 µg) was processed as described in the text. Electrophoresis was carried out in a 1% agarose gel (Seakem; FMC) with 45 mM Tris–borate–EDTA buffer (pH 8.0) by using a CHEF mapper system (Bio-Rad) at 14 °C for 22 h. Running conditions: voltage gradient 6 V/cm; included angle 120 °; linear switch time ramp 07–45 s. Lane M, DNA size markers (concatemeric lambda DNA; New England Biolabs). Asterisk, position of full-length EsV-1 DNA. The gel was imaged as described previously.

Fig. 3. Identification of virus sequences in algal DNA isolated from the sources illustrated in Fig. 1. Samples of EsV-1 DNA (a) and cellular DNA from PAr 10n (b), NZVicZ14 (c) and Nap R-B1 (d) were subjected to pulsed-field gel electrophoresis. The 1% low-melting-point agarose gel was run under the following conditions: voltage gradient 6 V/cm; included angle 120 °; linear switch time ramp 12–83 s; temperature 14 °C; running time 23 h. Ethidium bromide-stained DNA lanes (1) were cut into 0.5 cm slices for PCR analysis (2) as described in the text. The results were digitized (see legend to Fig. 1).

References


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