Figwort Mosaic Virus DNA Replicates in Cultured Datura stramonium Cells

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SUMMARY

Cellular forms of the DNA of the caulimovirus figwort mosaic virus (FMV), isolated from Datura stramonium leaves and callus cells derived from FMV-infected leaf tissue, have been studied. One- and two-dimensional gel electrophoresis and blot hybridization experiments showed the presence of various truncated forms of FMV DNA reminiscent of the putative replication intermediates of cauliflower mosaic virus (CaMV) thought to be generated by a mechanism involving reverse transcription. FMV DNA was found to be qualitatively and quantitatively similar in leaf and callus tissue, suggesting that, in contrast to CaMV, FMV can multiply efficiently in actively proliferating cells cultured in vitro.

The caulimoviruses are a unique group of plant viruses that encapsidate a genome of double-stranded DNA (see Shepherd, 1979; Covey, 1985). Most of our knowledge of the multiplication cycle of caulimoviruses comes from studies of one member of the group, cauliflower mosaic virus (CaMV). Considerable evidence is now available which supports the view that CaMV DNA is replicated by reverse transcription of an RNA intermediate (see Hull & Covey, 1983a; Hohn et al., 1985; Howell, 1985). This conclusion is based in part upon observations of putative replication intermediates isolated from CaMV-infected leaf tissue (Covey et al., 1983; Hull & Covey, 1983b; Turner & Covey, 1984; Marco & Howell, 1984; Marsh et al., 1985) and from protoplasts infected with CaMV in vitro (Maule, 1985; Thomas et al., 1985).

Aspects of CaMV multiplication have also been studied in in vitro propagated callus tissue derived from virus-infected protoplasts (Paszkowski et al., 1983) and leaves (Rollo & Covey, 1985). A significant reduction in the amount of CaMV DNA was observed during the early stages of callus culture with a concomitant change in the composition of subcellular viral DNA forms, which suggests that the ability of callus to support CaMV replication is limited (Rollo & Covey, 1985; S. N. Covey & F. Rollo, unpublished observations).

We report here our analysis of the DNA forms of figwort mosaic virus (FMV), a caulimovirus which has a 7.8 kilobase pair (kbp) circular DNA genome with four single-strand discontinuities and infects plants of the Scrophulariaceae (Hull & Donson, 1982). We have isolated FMV DNA forms from infected leaves of Datura stramonium and from 3.5-month-old callus tissue obtained by in vitro culture of FMV-infected leaf explants. Our results show that, in contrast to CaMV, FMV can replicate efficiently in cells cultured in vitro.

D. stramonium leaves of 20-day-old plants were mechanically inoculated with FMV (a gift of J. Donson). Two to 3 weeks after inoculation, systemically infected leaves were removed and surface-sterilized as reported previously (Rollo & Covey, 1985). To obtain callus cultures, sterile leaf pieces (2 cm²) were placed on 1.5% agar containing M & S medium (Murashige & Skoog, 1962) supplemented with 2,4-dinitrophenol (1 mg/l) and kinetin (0.5 mg/l) and containing carbenicillin (300 µg/ml). Carbenicillin was present throughout the growth of the callus and
those calluses grown for longer than 3-5 months were subcultured onto fresh medium containing carbenicillin. Callus tissue was cultivated in a growth chamber at 23 ± 1 °C with a 16 h day of about 10000 lx light intensity.

Total nucleic acids were extracted from infected leaf and callus tissue using phenol-based mixtures as reported by Covey et al. (1983). Viral DNA was detected in leaf or callus tissue by spot hybridization as follows. Total tissue homogenate or purified nucleic acid was added to an equal volume of 1 M-NaOH and serial fivefold dilutions were made in 0.5 M-NaOH. Samples (5 μl) were spotted onto nitrocellulose and the remaining procedures were as described by Maule et al. (1983). Radioactivity associated with hybridized spots was detected by autoradiography or quantified by liquid scintillation counting. DNA was fractionated in 1.5% agarose slab gels by electrophoresis in 40 mM-Tris-acetate buffer pH 7.6, 2 mM-EDTA (neutral medium) or 30 mM-NaOH (denaturing medium) at 2 V/cm. Two-dimensional gel electrophoresis was performed as reported by Hull & Covey (1983b).

To detect supercoiled molecules, DNA was depurinated in gels and then blotted onto nitrocellulose as described by Rollo & Covey (1985). Blots were probed with a recombinant clone (pFM4) containing full-length FMV DNA (Donson & Hull, 1983) which had been labelled with $^{32}$P by nick-translation.

To determine whether FMV DNA could replicate in actively proliferating cells, explants taken from leaves of systemically infected D. stramonium plants were cultured in vitro. After 3-5 months of culture, the explants had produced abundant callus tissue (about 0.5 g callus per single explant). The callus tissue was greenish but not uniformly so; it was also moderately friable although our attempts to obtain a dispersed cell suspension in liquid M & S medium failed. Calluses produced by different explants were harvested, pooled and total nucleic acid extracted as indicated above. Individual calluses which were left in culture continued to grow and reached
Fig. 2. Southern blot following gel electrophoresis of total DNA isolated from FMV-infected *D. stramonium* leaf (b, d) or 3.5-month-old callus tissue (a, c). DNA was fractionated in a neutral (a, b) or alkaline (c, d) electrophoresis medium. (e) structure of FMV DNA according to Hull & Donson (1982). The symbols indicate that the four single-stranded interruptions and the single-stranded fragments are 7-8 (α), 3.8 (β), 2.0 (γ) and 2.0 (δ) kb in size.

a fresh weight of about 3 to 5 g after 6 months from the inception of culture. We observed that the original leaf tissue did not persist as green tissue but became brown and apparently moribund. This was separated from the *in vitro* generated callus and the latter alone was used for viral DNA analysis. FMV DNA was determined by spot hybridization of total callus or leaf tissue homogenate (about 5 mg fresh weight). It was observed (Fig. 1) that the amount of virus-specific DNA present in callus tissue after 3.5 months of culture was similar to that found in infected leaves. This result was confirmed when equal amounts (fresh weight) of purified callus or leaf DNA were spotted onto nitrocellulose instead of total tissue homogenate. In the latter case, liquid scintillation counting of blots showed that FMV DNA present in total callus DNA approached 90% of that present in leaf DNA preparations. Viral DNA was also present in callus cultured for 6 months but at a much reduced level compared with younger callus.

The nature of the FMV DNA forms isolated from callus and leaves was investigated by fractionating total cellular DNA in 1.5% agarose gels in the presence of neutral or alkaline electrophoresis medium. The DNA was blotted onto nitrocellulose and probed with nick-translated FMV DNA (Fig. 2). In neutral buffer, FMV DNA from 3.5-month-old callus (Fig. 2a) and leaves (Fig. 2b) separated into five major bands (f1 to f5). Although the overall pattern of bands was similar in both leaves and callus, minor differences were apparent including an
Fig. 3. Southern blot of total DNA isolated from FMV-infected *D. stramonium* 3.5-month-old callus (*a*, *b*) or leaf tissue (*c*). The DNAs were fractionated by two-dimensional gel electrophoresis and samples (*b*, *c*) were treated with HCl before blotting to reveal supercoiled molecules. The open arrows indicate the direction of migration of the DNA in neutral (N) or alkaline (A) medium.
additional callus component migrating more slowly than form 1 (f1) DNA and an increase in intensity of a component migrating slightly more slowly than form 5 (f5) DNA. In addition, a reduction in the relative abundance of f1 DNA and a minor band of more rapid mobility was observed in callus DNA compared with leaf DNA (Fig. 2).

The pattern of FMV DNAs in callus and in leaves was also found to be very similar when electrophoresis was performed in an alkaline medium which denatures double-stranded molecules to generate single strands (Fig. 2c, d). Five major single-stranded fragments were observed in both preparations with sizes of 7-8, 6-7, 3-8, 2-3 and 2-0 kb. A minor FMV DNA component at 5-8 kb was observed in the callus preparation (Fig. 2c) but not in that from leaves (Fig. 2d). Hull & Donson (1982) concluded that the 7-8 kb and 3-8 kb components correspond to the α and β strands respectively (see Fig. 2e) whilst the γ and δ strands co-migrate at 2-0 kb. The other major components of 6-7 kb and 2-3 kb are thought to be produced by strand breakage at the site of FMV virion DNA gaps (Hull & Donson, 1982), a phenomenon also observed in CaMV DNA (Hull & Covey, 1983).

In our previous studies of CaMV DNA forms isolated from turnip callus, we found that truncated viral DNAs were diminished and supercoiled DNA had preferentially accumulated compared with those isolated from leaves (Rollo & Covey, 1985). To examine FMV DNAs more closely, we fractionated them by two-dimensional gel electrophoresis as described by Hull & Covey (1983). This method permits the resolution of both supercoiled forms and specific single-stranded DNAs that associate in double-stranded molecules. From leaves, the ds f1 DNA was resolved into 7-8, 3-8 and 2-0 kb single-stranded fragments (Fig. 3c) and from its mobility compared with that of the open circular form of CaMV DNA in two-dimensional gels (Hull & Covey, 1983b) we conclude that this represents the open circular form of FMV DNA. Like that of CaMV, the open circular form (f1) of FMV sometimes shows multiple bands on gels due to the presence of twisted molecules, a pattern which differs slightly between leaves and callus (see Fig. 2a, b). This form was somewhat reduced in Datura callus (Fig. 3a, b) which showed a relative increase in the amount of the linear forms f2 and f3, suggesting that a greater proportion of open circular molecules had been linearized compared to leaf tissue. Furthermore, a DNA form co-migrating in the neutral dimension with f4 DNA and revealed only after a depurination step (compare Fig. 3a and b) represents the supercoiled form of FMV DNA which was slightly more abundant in callus (Fig. 3b) than in leaves (Fig. 3c).

The present report shows that when FMV-infected leaf tissue of D. stramonium is induced to proliferate callus by in vitro culture, the ratio of FMV DNA to cell DNA remains virtually unchanged for more than 3 months. Moreover, the structural forms of FMV DNA which are found in the leaf tissue are still present in the callus and only minor differences in their relative abundance can be observed. In a previous investigation (Rollo & Covey, 1985), we described the structural forms of CaMV DNA in leaf and callus tissue. From that study it clearly emerged that the replication pattern of CaMV DNA had undergone a dramatic alteration in cultured cells. In fact, the amount of cellular CaMV DNA was found to decrease suddenly after the first week of in vitro cultivation, reaching 20% of that found in leaf tissue. This phenomenon was followed by an equally dramatic change in the composition of CaMV DNA forms. The open circular, linear and truncated forms were found to decline in turnip callus whilst supercoiled DNA persisted and represented the bulk of unencapsidated CaMV DNA in this tissue. In contrast, supercoiled FMV DNA in Datura callus was found to be a relatively minor component of the unencapsidated DNA forms although it was more abundant in callus than in leaves.

The loss of truncated CaMV DNA forms and the decline in amount of total viral DNA in turnip callus suggested that these cells were not capable of supporting CaMV replication by reverse transcription. However, in Datura callus both the truncated forms and the quantity of total FMV DNA were maintained, from which we conclude that active viral DNA replication, presumably including reverse transcription, is taking place for at least 3.5 months in culture. This in vitro cell system should prove useful in studying aspects of caulimovirus DNA replication that are at present difficult using other methods currently available.
Short communication

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REFERENCES


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