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Characterisation of DNA forms associated with cassava latent virus infection

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ABSTRACT

In addition to the major encapsidated DNA species found in preparations of cassava latent virus (genomic DNAs 1 and 2) there are minor DNA populations of twice (dimeric) and approximately half genome length. Both minor species resemble the genomic DNAs in that they are composed of predominantly circular single-stranded DNA. All of these size groups have a corresponding covalently-closed circular double-stranded DNA form in infected tissue. Infectivity studies using cloned DNAs 1 and 2 show that dimeric DNA routinely appears, suggesting it to be an intermediate in the DNA replicative cycle that can be encapsidated at low efficiency. In contrast, half unit length DNA has not yet been detected after multiple passaging of virus derived from the cloned DNA inoculum. Half unit length DNAs appear to be derived exclusively from DNA 2 and consist of a population of molecules exhibiting a relatively specific deletion. As they have an inhibitory effect on virus multiplication, their encapsidated forms are analogous to defective interfering particles associated with other eukaryotic DNA containing viruses. Small primer molecules associated with the genomic single-stranded DNAs, as reported for another geminivirus, have not been detected in CLV.

INTRODUCTION

Geminiviruses are characterised by a genome of circular single-stranded (ss) DNA encapsidated in twinned (geminate) particles. While a number of whitefly-transmitted geminiviruses have been demonstrated to have a bipartite genome (bean golden mosaic virus (BGMV) (1), cassava latent virus (CLV) (2,3) and tomato golden mosaic virus (TGMV) (4,5)), the genome of the leafhopper-transmitted geminivirus maize streak virus (MSV) has so far been shown to comprise only a single DNA (6,7). The mechanism of geminivirus DNA replication is poorly understood at the present time; double-stranded (ds) DNAs, presumed to be intermediates in the replicative cycle, have been isolated from plants infected

with BGMV (8), TGMV (9) and MSV (7). In addition to the open circular (oc) DNA reported for BGMV, both TGMV and MSV show covalently-closed circular (ccc) DNA. Recently, a small DNA fragment containing a number of 5' terminal ribonucleotides has been shown to be complementary to and associated with encapsidated MSV DNA (6,10) and has been implicated in the process of second strand synthesis.

Examination of the DNA from purified geminate particles prepared from a native isolate of CLV shows, in addition to the two genomic DNAs, small amounts of DNA of approximately twice and half genomic DNA length (3). In this report, these minor encapsidated DNAs are characterised and related to dsDNAs isolated from CLV-infected tissue. The results are discussed in terms of DNA replication and encapsidation.

MATERIALS AND METHODS

Virus purification and extraction of encapsidated DNA

The Kenyan isolate of CLV was mechanically inoculated to Nicotiana benthamiana maintained at 25°C. CLV was purified as described by Sequeira and Harrison (11). Virions of the Nigerian isolate of MSV, purified from Zea mays infected by viruliferous leafhoppers, were a gift from M. Boulton. DNA was extracted from virions by phenol extraction in the presence of 0.1% SDS and precipitated with ethanol. DNA was redissolved to a concentration of 1 mg ml^{-1} in water.

Preparation of DNA probes specific to CLV DNAs 1 and 2

Replicative forms of M13 clones containing full-length copies of DNAs 1 (pJS076) or 2 (pJS023) were double-digested with either AhaIII and MluI (DNA 1) SphI and MluI (DNA 1), HpaI and EcoRI (DNA 2) or EcoRV and EcoRI (DNA 2). Digests were fractionated on a 1% agarose gel (low melting point agarose, Bethesda Research Laboratories), stained with ethidium bromide and appropriate fragments excised and eluted as described (12). Fragments specific to DNA 1 spanned AhaIII (221) to MluI (734) and MluI (734) to SphI (2581) and those specific to DNA 2 spanned HpaI (416) to EcoRI (1528) and EcoRI (1528) to EcoRV (2550). Aliquots of encapsidated DNA (200 ng) were fractionated on a 1.4% agarose gel, Southern blotted (13) and probed with each of the above

nick-translated fragments.

Characterisation of encapsidated DNA

A mixture of 200 ng CLV encapsidated DNA and 200 ng ClaI-linearised pAT153 in a total of 10 µl was digested with either 10 ng DNase 1 in 10mM Tris HCl (pH 7.5), 10mM $MgCl_2$ for 30 min at 37°C or 6 units S_1 nuclease in 280mM NaCl, 30mM Na acetate, 4.5 mM Zn acetate (pH 4.4) for 30 min at 20°C. Digests were fractionated on a 1.4% agarose gel and bands visualised by staining with ethidium bromide. Gels were Southern blotted (13) and probed with nick-translated M13 RFs containing full-length DNAs 1 and 2 (pJS023 and pJS076). Alternatively, 200 ng CLV encapsidated DNA was fractionated on an alkaline 1.4% agarose gel in 30 mM NaOH, 2mM EDTA and subsequently blotted and probed as above.

Comparison of CLV and MSV encapsidated DNAs

CLV and MSV encapsidated DNAs were pretreated with calf intestine phosphatase and end-labelled using polynucleotide kinase as previously described (10). Labelled DNA was analysed on a denaturing 8% polyacrylamide gel (14) or by two-dimensional agarose gel electrophoresis as described by Favaloro et al (15). Alternatively, 200 ng aliquots of encapsidated DNA were fractionated by two dimensional agarose gel electrophoresis (15), Southern blotted (13) and probed with nick-translated M13 RFs containing full-length CLV DNAs 1 and 2 (pJS023 and pJS076) or full-length MSV DNA (7) as appropriate. To investigate the potential self-priming capability of the CLV encapsidated DNA, DNA polymerase-directed second strand synthesis was carried out as described (10).

Purification of CLV cccDNA

Systemically infected leaf material was frozen in liquid nitrogen and ground to a fine powder. The material was suspended in two volumes of 10mM Tris HCl (pH 7.0), 100mM NaCl, 10mM EDTA, 1% SDS, extracted three times with an equal volume of phenol/chloroform (8:2 v/v) and nucleic acids precipitated with ethanol. The precipitate was redissolved in 100mM Tris HCl (pH 7.4), 10mM EDTA, 100µg ml^{-1} ethidium bromide, an equal weight of CsCl added and cccDNA purified by two cycles of CsCl gradient centrifugation. The DNA was extracted four times with isobutanol saturated with water and CsCl, dialysed extensively against water,

precipitated with ethanol and finally redissolved in water to a concentration of 1 mg ml^{-1}. Prior to electrophoretic analysis, cccDNA samples were treated with S_1 nuclease (5u μg^{-1} DNA) in 280mM NaCl, 30mM Na acetate, 4.5mM Zn acetate (pH 4.4) for 30 min at 4°C to remove traces of contaminating CLV ssDNA.

Analysis of dimeric cccDNA

Purifed cccDNA was fractionated on a 1.4% low melting point agarose gel, stained with ethidium bromide and bands corresponding to genomic and dimeric cccDNAs eluted (12). Aliquots of the DNAs were digested with MluI or PstI and the products separated on a 1.4% agarose gel. The gel was Southern blotted (13) after an initial depurination step to ensure efficient transfer of ccc DNA (16) and probed with nick-translated M13 RFs containing inserts specific to either DNA 1 (pJS049, BglII (804) to BglII (2061)) or DNA 2 (pJS055, HindIII (1494) to HindIII (2281)).

Cloning and sequencing of defective DNA

Purified cccDNA was digested with either PstI or SalI and the products fractionated on a 1.4% low melting point agarose gel. Linearised defective DNA was located by ethidium bromide staining and eluted as described (12). DNA was cloned into M13 mp9 linearised with the appropriate restriction enzyme. The sequences of the termini of the DNA inserts were established using the dideoxy-termination procedure in either the forward (12) or reverse sense (17). The complete sequence of defective DNA clone pDEF004 was established after subcloning HindIII, SalI, Sau3A and TaqI fragments in M13mp9.

Infectivity studies

M13 RFs containing full-length copies of DNAs 1 (pJS092) and 2 (pJS094) were digested with MluI and PstI respectively to excise the inserted DNA and the digests phenol extracted and precipitated with ethanol. DNA was redissolved in a concentration of 1 mg ml^{-1} with respect to the DNA insert. A full-length copy of a defective DNA was excised from M13 RF pDEF004 using PstI and processed as described above, with or without prior treatment with DNase 1 at a concentration of 10µg ml^{-1} for 30 min at 37°C. Routinely 1µg each of cloned DNAs 1 and 2 in 10µl 100mM Na phosphate (pH 7.0) together with varying amounts of cloned defective DNA were mechanically inoculated to two week old N. benthamiana

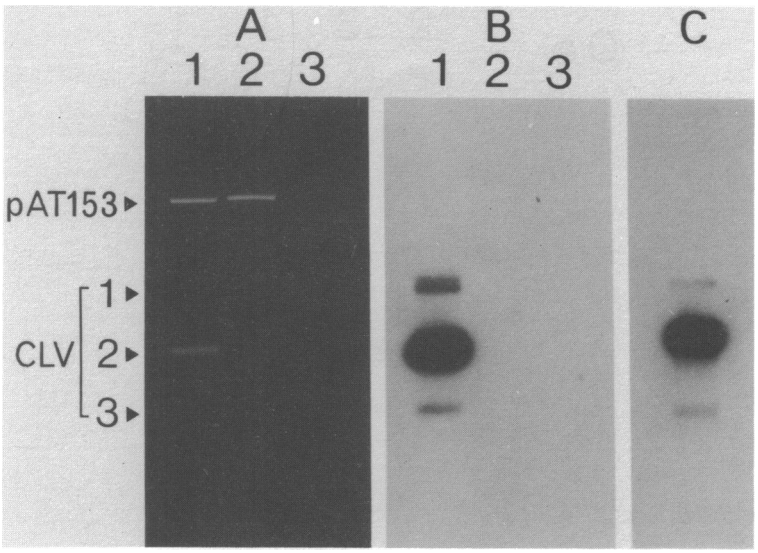

Figure 1. Ethidium bromide stained non-denaturing agarose gel (A) and its Southern blot (B) of encapsidated CLV DNA treated as follows: lane 1, no treatment; lane 2, S_1 nuclease; lane 3, DNase 1. A Southern blot of the DNA on an alkaline agarose gel is shown (C). The position of linearised pAT153, used as a control in the nuclease digestion experiments, is indicated.

maintained at 25°C. Symptoms were allowed to develop for a period of up to 20 days post inoculation.

Electron microscopy

Samples of encapsidated and cccDNA were spread at 50°C as described by Robinson et al (18).

RESULTS

Analysis of encapsidated DNA

When purified encapsidated DNA from a native isolate of CLV is fractionated by agarose gel electrophoresis under non-denaturing conditions, one major band corresponding to genomic DNAs and three minor bands are routinely observed (3). The nuclease susceptibility data of Figure 1 show that all encapsidated nucleic acids are completely degraded by both DNase 1 and S_1 nuclease but are not hydrolysed in the presence of alkali inferring that the material contained within the minor bands, like the genomic DNAs, is composed of ssDNA. The doublet band 1 seen under

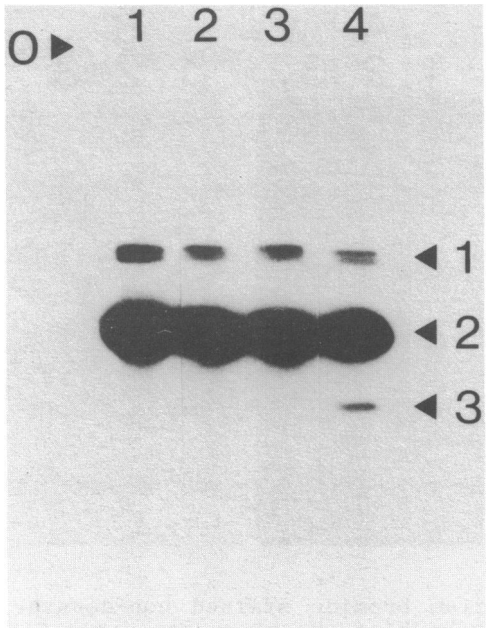

Figure 2. Southern blot of encapsidated CLV DNA. The nucleotide coordinates of the fragments used as probes are as follows: lane 1, DNA 1 AhaIII (221)- MluI (734); lane 2, DNA 1 MluI (734)- SphI (2581); lane 3, DNA 2 HpaI (416)- EcoRI (1538); lane 4, EcoRI (1528)- EcoRV (2550).

non-denaturing conditions (Figure 1B) appears as a single band on an alkaline gel (Figure 1C), suggesting that one component of the doublet is composed of non-covalently bound dimers of the genomic DNAs. This suggestion is substantiated by the data of Figure 4, in which the linear form of these non-covalently associated dimers is seen to be disrupted to produce linear genomic DNA under denaturing conditions. When hybridised with DNA-specific probes (Figure 2) bands 1 and 2 appear in every case whereas band 3 is observed only when probed with DNA 2 fragment EcoRI (1528)- EcoRV (2550), confirming the suggestion that the material is specific to DNA 2 (3) and mapping the deletion within the defective molecule.

CLV genomic DNAs consist of a mixture of circular and linear molecules (20), the latter probably arising from degradation of the circular form. To observe the configuration of the minor

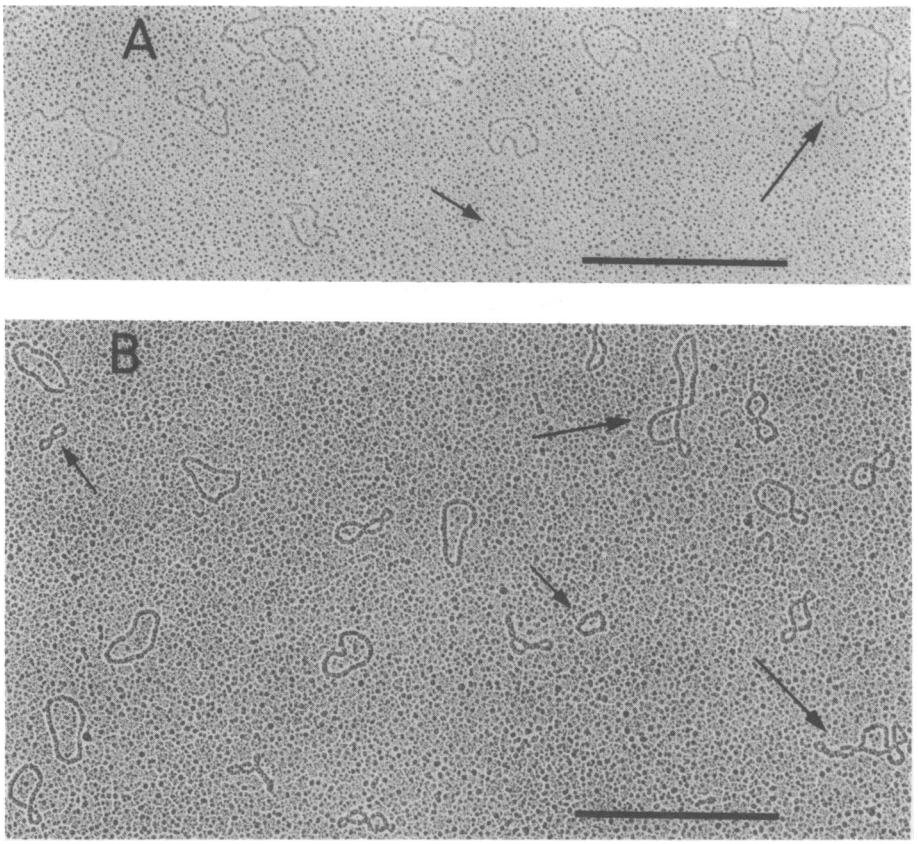

Figure 3. Electron micrograph of encapsidated CLV DNA (A) and cccDNA from CLV-infected tissue (B). The positions of dimeric (large arrows) and defective DNA (small arrows) are indicated. The bar represents 1μm.

components of the encapsidated DNA, DNA spreads were examined by electron microscopy for the presence of molecules other than genomic DNAs. The electron micrograph of Figure 3A has been selected to show, in addition to genomic size circular molecules, both dimeric (large arrow) and defective (small arrow) circular DNA molecules.

Comparison of CLV and MSV encapsidated DNAs

Unlike CLV, only a single genomic DNA has been found associated with MSV infection (6,7). Furthermore, a small DNA fragment containing 5' terminal ribonucleotides and complementary to the

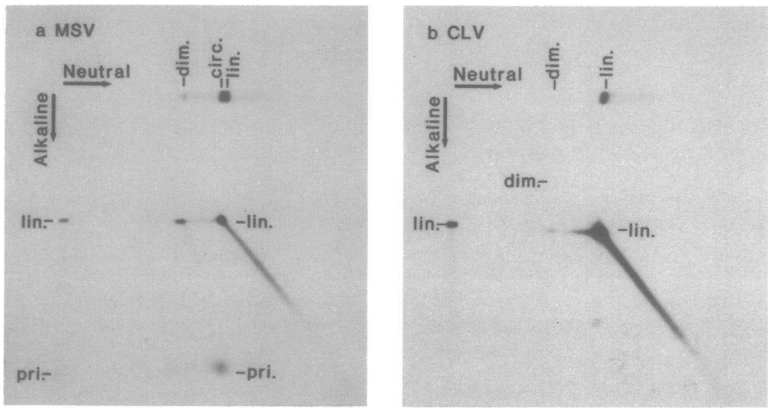

Figure 4. Two dimensional agarose gel electrophoresis of 5' terminally-labelled encapsidated DNA from MSV (a) and CLV (b). The positions of dimeric (dim) DNA, circular (circ) and linear (lin) forms of genomic DNA and the MSV primer (pri) are indicated.

genomic DNA has been shown to be encapsidated within MSV geminate particles (6,10). It has been suggested that the fragment serves as a primer for second strand synthesis. In an attempt to find an analogous fragment within encapsidated CLV DNA, the latter was terminally-labelled and fractionated as shown in Figure 4. In the neutral dimension, labelled MSV DNA separates into three major species, namely dimeric DNA and circular and linear forms of genomic DNA. Although only the linear forms of both the dimeric and genomic DNAs would be expected to become 5' terminally labelled, the circular forms remain associated with the labelled primer under the non-denaturing conditions and are consequently seen on the autoradiograph. The primer becomes dissociated from these molecules in the alkaline dimension to give a clearly visible product at the bottom of the gel. The MSV dimeric DNAs resemble those of CLV, being composed predominantly of non-covalently associated genomic DNAs together with true dimeric molecules which, although they cannot be seen in Figure 4 for either MSV or CLV, become evident after a longer period of autoradiography. In contrast to MSV, only a single genomic DNA band, corresponding to 5' terminally labelled linear molecules, is seen in the neutral dimension. Furthermore, no product analogous to the MSV primer molecule is evident on dissociation of

the labelled material in the second dimension, even after extended autoradiography. Although the MSV fragment is approximately 80 nucleotides in length it is possible that a smaller CLV fragment may be overlooked in Figure 4. Both CLV and MSV terminally -labelled DNAs were therefore run on an 8% denaturing gel under conditions in which a fragment with a minimum size of approximately 20 nucleotides would be detected. While the MSV fragment appeared as a multiple band migrating at the expected position (10), no significantly labelled CLV products were visible (data not shown). To eliminate the possibility that a putative CLV fragment has a blocked 5' terminus and is consequently not amenable to labelling with polynucleotide kinase, encapsidated CLV and MSV DNAs were fractionated by two dimensional gel electrophoresis, blotted and probed with appropriate nick-translated cloned genomic DNAs. Again, although the MSV fragment was clearly visible an analogous CLV product was not detected (data not shown). Finally, the potential for DNA polymerase-directed second strand synthesis using CLV ssDNA template was investigated. The self-priming activity of MSV virion DNA producing full-length ds DNA has been described (10). In a parallel experiment no appreciable level of radioactivity was incorporated into CLV DNA as judged by the production of full-length dsDNA either as the open circular or linear form (the latter produced after treatment with single cutting restriction enzymes) (data not shown).

Analysis of cccDNA

When examined by electron microscopy, cccDNA preparations show predominantly circular genome length dsDNA (Figure 3B). Minor populations of twice (large arrows) and half (small arrows) genome length dsDNA are also present.

When fractionated by agarose gel electrophoresis cccDNA prepared from CLV-infected material gave the banding pattern shown in Figure 5, lane 1. The slow migrating material (larger than 7,200 base pairs) is not virus-specific (as shown by Southern blotting) and corresponds to host DNA contamination of the ccc DNA. The virus-specific material has been arranged into three size groups on the basis of the restriction enzyme analyses of lanes 2 to 5 together with a knowledge of the nucleotide sequence (2). Genomic cccDNA together with its linear and ocDNA coun-

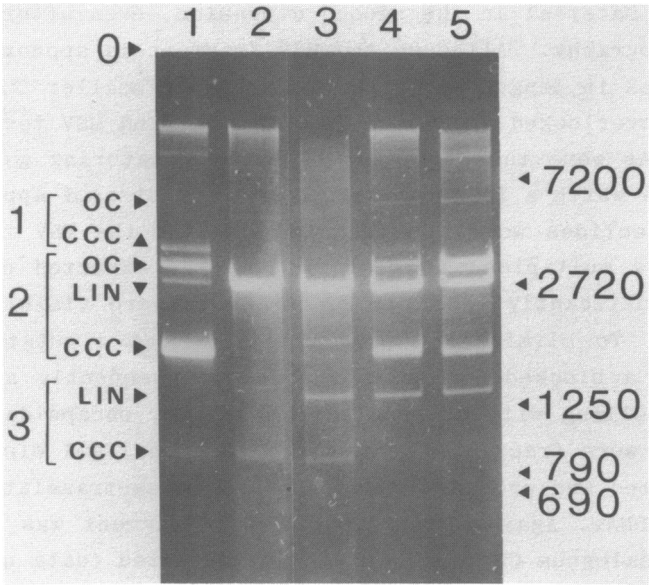

Figure 5. Ethidium bromide stained agarose gel of CLV cccDNA treated as follows: lane 1, no treatment; lane 2, ClaI; lane 3, EcoRV, lane 4, SalI; lane 5, PstI. Size marker numbers refer to base pairs.

terparts (group 2) accounts for the majority of the virus-specific DNA. The presence of linear and ocDNA forms are due mainly to the S_1 nuclease step introduced prior to electrophoresis to remove ssDNA contaminants. S_1 nuclease sensitive sites have been described for TGMV (21), and in CLV are most likely located within the region common to DNAs 1 and 2 at the position of the most stable hairpin structure (2). The genomic cccDNA is completely linearised with ClaI (lane 2) and EcoRV (lane 3), both enzymes having a single site on both DNAs 1 and 2. Two extra bands with estimated sizes of 1,800 and 800 base pairs (lane 3) are due to a minor population within the native isolate of CLV that contains a second EcoRV site. As both SalI (lane 4) and PstI (lane 5) have a single site only in DNA 2 and are not represented in DNA 1, only a proportion of the genomic cccDNA is linearised.

Group 1 cccDNA (Figure 5) together with genomic cccDNA was extracted from agarose for further analysis. The results displ-

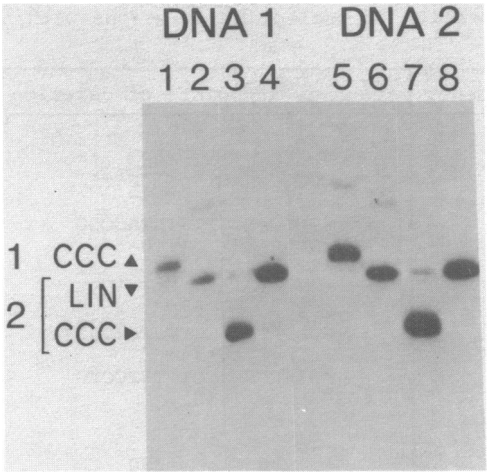

Figure 6. Southern blot of genomic (lanes 3,4,7 and 8) and dimeric (lanes 1,2,5 and 6) cccDNAs treated as follows: lanes 1,3,5 and 7, no treatment; lanes 2 and 4, MluI; lanes 6 and 8, PstI. Lanes 1-4 and 5-8 were probed with nick translated DNA specific to DNAs 1 and 2 respectively.

ayed in Figure 6 show that most of the DNA 1-specific cccDNA is linearised with MluI and DNA 2-specific cccDNA is linearised with PstI. MluI and PstI have single sites specific to DNAs 1 and 2 respectively. Because a small amount of genomic cccDNA remains undigested (lanes 4 and 8) it is thought that the faint bands in lanes 2 and 6 represent partial digestion products of dimeric cccDNA. The major form of linearised group 1 cccDNA comigrates with that from genomic cccDNA, confirming that this material is composed of a mixture of dimeric forms of DNAs 1 and 2.

When Southern blotted and probed with the DNA-specific fragments described in the legend to Figure 2, group 3 DNA (Figure 5 hybridises only with the DNA 2 probe encompassing EcoRI (1528)-EcoRV (2550) (data not shown), and so represents the double-stranded form of the defective ssDNA. When treated with restriction enzymes that have a single site within DNA 2, the defective ds DNA remains undigested with ClaI (1095) (Figure 5, lane 2) but is linearised with EcoRV (2550), SalI (1753) and PstI (245) (lanes 3-5 respectively). Together with a size estimate for the

Table 1. Location of nucleotide deletions within DNA 2.

type	frequency	sequence at points of deletion	DNA length
1	1	190 200 CCCGCGCACT*GGTTGGCTTC* •• *ATCTCATG*CTTGTATAGCGC 1600 1610	1315
2	1	270 280 GCATATAGATGTT*GTCAGCT* ••••• *TGTAGCCC*ATGTTTCTCCTG 1630 1640	1363
3	1	280 290 GATGTTGTCAGC*TATGGATA* ••• *TTTTGAATG*AGCCTTCGAAT 1710 1720	1294
4	16	340 350 GGGCCAATCGAATG*ACAGCT* ••••••• *TGAGCCT*TCGAATGGGCTTA 1720 1730	1343

linearised group 3 DNA of 1300 nucleotides, the results are consistent with a deletion of approximately 1,400 nucleotides from DNA 2 localised downstream of the PstI site (245) and upstream of the SalI site (1753). To define the deletion points more precisely, PstI- and SalI-linearised group 3 dsDNA was eluted from agarose and cloned into M13mp9. The sequences at the termini of the inserts, which should be within 100-200 nucleotides of the deletion, were determined by the dideoxy-termination procedure. In Table 1, the nucleotides adjacent to the deletion points have been aligned to show small repeat sequences present in all clones so far examined. In addition to the nucleotides shown in italics, one copy of each repeat is deleted. Of the 19 clones examined, 16 showed the same deletion (Table 1, type 4) and all clones except type 1 contained both the PstI and SalI sites. The type 1 clone was deleted upstream as far as the 3' terminus of the region common to DNAs 1 and 2. One example of the type 4

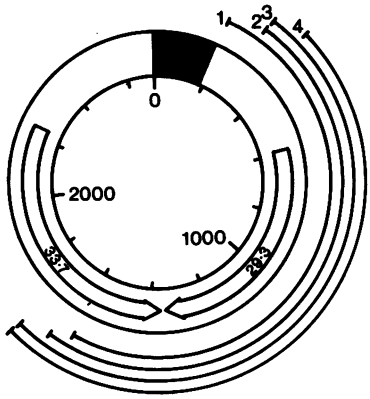

Figure 7. CLV DNA 2 showing the extent of the nucleotide deletions relative to the region common to DNAs 1 and 2 (2) (stippled area) and the major open reading frames with potential coding capacities of 33.7 and 29.3 kilodaltons (19). Deletions 1 to 4 correspond to those shown in detail in Table 1.

clone, pDEF004, was completely sequenced to ensure that no other insertions or deletions had occurred. The results show that in addition to 10 nucleotide substitutions dispersed throughout the DNA, a single T residue had been inserted within the three T residues located between nucleotides 96-98 (2), positioned within the region common to DNAs 1 and 2. The extent of the deletion in the four clone types is shown in Figure 7 in relation to the two major open reading frames of DNA 2 and the region common to DNAs 1 and 2.

Infectivity in the presence of defective DNA

While both genomic and dimeric DNAs can be extracted from purified virus when cloned CLV DNAs 1 and 2 are used to infect N. benthamiana, defective DNA is not present (3). Five passages of systemically infected material from the original cloned DNA inoculum have failed to produce this DNA. The defective DNA is therefore non-essential to CLV multiplication.

The infectivity of various DNA combinations was assayed using N. benthamiana as host and screening for primary symptom development (lesions on inoculated leaves) and systemic spread of virus (concomitant with severe leaf curling symptoms). Ideally, infectivity studies of this nature would be more quantita-

Table 2
Infectivity of CLV DNAs 1 and 2 in N. benthamiana in the presence of varying amounts of defective DNA

defective DNA (μg leaf^{-1})	infected/inoculated plants exp. 1	exp. 2	exp. 3	exp. 4
-	5/5	5/5	5/5	5/5
0.02	nd	5/5	5/5	nd
0.1	4/5	5/5	3/5	nd
0.5	nd	5/5	3/5	nd
1	0/5	nd	2/5(5/5)	3/5(5/5)
3	nd	nd	nd	0/5(5/5)
10	0/5	nd	nd	0/5(3/5)

Bracketted results refer to defective DNA pretreated with DNase 1.
nd = not done.

tive using a local lesion host. However, although Datura stramonium has been suggested as a possible local lesion host (22), attempts to infect plants with cloned DNAs 1 and 2 have so far been unsuccessful. The results summarised in Table 2 show that when the concentration of defective DNA is increased within the inoculum containing DNAs 1 and 2, there is a decrease in the number of plants showing symptoms. Furthermore, there is a direct correlation between the amount of defective DNA in the inoculum and the time required for symptom development. When the defective DNA is treated with DNase 1, the infectivity of the inoculum is restored to a level approaching that in the absence of the defective DNA, inferring that the inhibitory effect on symptom development is due to the DNA itself and not a contaminant within the DNA preparation. Encapsidated defective ssDNA can be recovered from leaf material showing systemic infection after inoculation using a mixture of cloned DNAs 1 and 2 and defective DNA. Inocula containing cloned DNA 1 and defective DNA 2 in varying amounts did not induce symptoms or result in the systemic movement of virus (as judged by dot blotting potentially infected leaf material).

DISCUSSION

Two minor circular ssDNA species of approximately twice and half genomic length DNA are encapsidated with CLV DNAs 1 and 2.

In contrast to the situation for MSV (6,10), an exhaustive search has failed to detect a small primer-like DNA fragment encapsidated with the genomic DNA. Double-stranded DNAs of similar size to the minor species have been isolated from CLV-infected tissue. As the larger of two dsDNAs consists of a mixture of dimeric forms of both DNAs 1 and 2, it is believed that the larger encapsidated species represents dimeric genomic ssDNA. One may envisage these single-stranded molecules arising from inefficient DNA cleavage during a rolling circle mechanism of DNA replication (23). Available data concerning the geminiviruses chloris striate mosaic virus (24) and BGMV (25) suggest that each geminate particle contains a single genomic ssDNA molecule. As dimeric ssDNA can be encapsidated it infers that either CLV geminate particles are not size limited to a single copy of either DNA 1 or 2 or that dimeric ssDNA is encapsidated in larger virion structures. It might be expected that a dimeric molecule would require a tetrameric virion morphology similar to that observed within preparations of the geminivirus squash leaf curl virus (26). As there are no major inverted repeat sequences either within or between each genomic ssDNA, it is difficult to understand why the bulk of the DNA within the non-denatured chimeric DNA population is composed of non-covalently associated molecules. The suggestion that residual proteins may be responsible (3) remains valid.

The smaller of the two minor encapsidated ssDNA species has a double-stranded counterpart within CLV-infected material. Southern blotting has demonstrated that the DNA originates from DNA 2 and sequence analysis of cloned copies has precisely located a set of deletions within one half of the molecule (Figure 7). All deletions disrupt both major open reading frames (ORFs) of DNA 2, completely excising the 29.3Kd ORF and removing the C terminal half of the 33.7Kd ORF. The deletions presumably arise during DNA replication although the mechanism is not understood. Interestingly, small repeat sequences are found at the deletion points of all four characterised defective types (Table 1) which may contribute to the exact location of the recombination event. While it is likely that other defective DNAs are produced during replication of the genome, only those DNAs that can be replica-

ted will be propagated within the virus population. Thus, the origin(s) of replication must be located within those sequences of DNA 2 that are conserved within the defective molecules. It may be significant that all four defective types have retained the sequence common to DNAs 1 and 2, suggested as playing a role in DNA replication (2). Furthermore, as the defective molecules are encapsidated, any signals necessary for this process must be retained within the defective DNA. Assuming that no additional major insertions or deletions have occurred in the defective molecules, as suggested from the nucleotide sequence of one defective DNA clone, the data of Table 1 suggest a relatively stringent size selection mechanism is imposed on the defective molecules. In view of their size being approximately half that of the genomic DNAs, the individual defective molecules may be encapsidated within isometric particles often seen in geminivirus preparations. This suggestion is consistent with the observation that preparations of isometric particles are enriched in defective DNA (27). Small virus-specific DNAs, suggested as being defective molecules have been isolated from plant tissue infected with TGMV (4,9). In addition to having a discrete size (although with an estimated size larger than the CLV counterparts) the TGMV defectives probably arise solely from component B (analogous to CLV DNA 2) (4). The reason for the absence of CLV DNA 1 or TGMV DNA A defective molecules is unclear but must be a reflection of the stringent size selection (possibly for encapsidation) in combination with the requirement for DNA replication in the presence of helper virus. If this proves to be the case, it should be possible to construct defectives from cloned CLV DNA 1 using selected parts of the molecule, in order to help clarify our understanding of these processes. The infectivity data of Table 2 show that the presence of a sufficiently high level of defective DNA within the inoculum of DNAs 1 and 2 impedes virus multiplication within the host. In this respect, the encapsidated defective molecules resemble the defective interfering (DI) particles of animal DNA viruses (reviewed in 28). It is essential that a selective advantage is conferred on DI particles over helper virus, often at the level of DNA replication or possibly DNA encapsidation, to maintain their numbers

within the virus population. Whether or not the deletions within DNA 2 make a replicative origin more accessible to a polymerase (there is certainly no duplication of a replicative origin as seen for some DI particles (28)) or the smaller molecules can be more easily encapsidated remains to be seen.

ACKNOWLEDGEMENTS

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