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Nuclei purified from cauliflower mosaic virus-infected turnip leaves contain subgenomic, covalently closed circular cauliflower mosaic virus DNAs

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ABSTRACT

Nuclei isolated from cauliflower mosaic virus (CaMV) infected turnip leaves contain subgenomic CaMV DNA species in addition to the genome length CaMV DNA. These subgenomic CaMV DNA species are present as covalently closed circles (form I), relaxed circles (form II) and linear (form III) molecules. The subgenomic form I DNA species range in size from about 10% of genome length to nearly genome length. These subgenomic DNA species appear in tissue infected with cloned CaMV DNA, indicating that they arise rapidly and have not accumulated in the virus population from serial propagation of CaMV. No specific region of the CaMV genome appears to be preferentially deleted to form the subgenomic CaMV DNA species. At least three distinct subgenomic species appear to accumulate preferentially in nuclei isolated from infected tissue. Two of these abundant subgenomic CaMV DNA species are form I and the other one is form III. Some of the subgenomic CaMV DNA species appear to be minichromosomes.

INTRODUCTION

Cauliflower mosaic virus (CaMV) is a plant virus containing a double-stranded DNA genome of approximately 8 kilobases (for reviews see 1-4). The DNA isolated from purified virus particles is circular, but not covalently closed; instead, it contains 3 site-specific discontinuities (5,6). One discontinuity is in the α-strand at map position 0 and the other two are located in the complementary strand at 0.20 and 0.53 map units on the circular genome (Fig. 1). The site-specific discontinuities are not nicks or gaps, but are triple-stranded structures where one strand overlaps itself by 8-43 bases (7). The position of the 5'-end of the overlap is constant while the 3'-end location is variable (7,8).

Nuclei isolated from CaMV-infected turnip leaves contain a transcriptionally active minichromosome of covalently closed circular (form I) CaMV DNA (9-12). The CaMV minichromosome is transcribed by RNA polymerase II. This transcription occurs only from the α-strand of the CaMV DNA, which is the strand transcribed in isolated nuclei (13) and in vivo (14). Three major polyadenylated CaMV transcripts and a heterogeneous group of RNA species (approximately 23S) have been identified in infected tissue (15-20). One major transcript (19S RNA) codes for

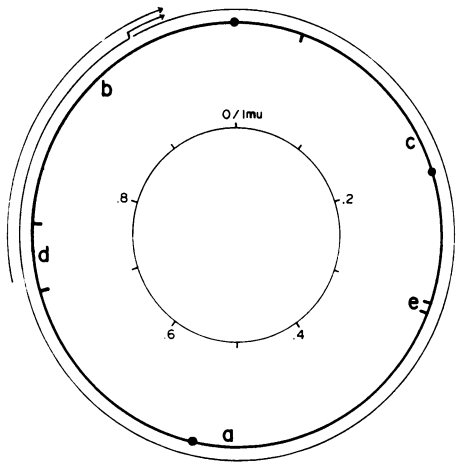

Figure 1. Map of CaMV genome. Inner circle shows map units (mu). The other circle shows the position of the site-specific discontinuities (—•—), and Eco RI restriction sites (—|—; labeled a-e). The major 35S and 19S transcripts are shown by arrows (5'——→3').

the inclusion body protein. The other transcripts are 35S RNA, a greater than genome length transcript (genome length plus 180 nucleotides) and 8S RNA, a noncoding 610-680 nucleotide transcript. The positions of the 5'- and 3'-ends for the 19S and 35S transcripts have been mapped (18-20); their positions are indicated in Figure 1.

It has been proposed that CaMV replicates by reverse transcription (21-23). The proposed mechanism is similar to that of retroviruses (for review see 24) and hepatitis B virus (25). The evidence for this model can be summarized as follows: a.) the 35S CaMV transcript contains a terminal 180 base pair direct repeat which could allow reverse transcriptase to synthesize a circular DNA copy of CaMV, b.) the 35S transcript has a site at map position 0 where $tRNA^{Met}$ could hybridize and act as a primer for replication, c.) linear subgenomic CaMV DNA species found in infected tissue (21,26,27) can be explained as intermediates of replication, d.) the site-specific discontinuities with triple-stranded overlaps may be the result of initiation of DNA synthesis at a specific location followed by strand displacement at the end of synthesis, and e.) a CaMV DNA synthesis activity with properties which suggest replication on a mixture of DNA and RNA templates is present in CaMV-infected tissue (11,23).

In this paper we extend a preliminary report that isolated CaMV-infected turnip leaf nuclei contain subgenomic species of CaMV DNA (11). The subgenomic

CaMV DNA species are present as form I, relaxed circular (form II) and linear (form III) DNA. We discuss possible mechanisms by which these species could arise and possible functions of specific subgenomic species.

METHODS

Propagation of CaMV and isolation of nuclei and CaMV minichromosome. CaMV was propagated in turnip leaves (Brassica rapa C. V. Just Right). Plants were infected with either CaMV (isolate CM1841-Mn) or with virus derived from plants which had been infected according to Howell et al. (28) with a genomic clone of CM1841-Mn (pMnS-4). CM1841-Mn was isolated from leaf tissue which had been infected with CM1841 and propagated serially at the University of Minnesota, St. Paul. CM1841-Mn has the same restriction map as CM1841, except that the 0.40 map unit Hind III site is absent in CM1841-Mn. Turnip leaf nuclei and the CaMV minichromosome were isolated as described previously (9). Minichromosome DNA was deproteinized by phenol extraction in the presence of 1% Sarkosyl (9).

Isolation of subgenomic CaMV DNA species. Nuclei were isolated from deveined turnip leaves, lysed, and turnip chromatin was removed by centifugation (9). The supernatant was adjusted to 1% Sarkosyl and 100 µg/ml proteinase K (E. Merck), incubated at 50°C for 3 h, and phenol extracted. The nucleic acids were precipitated with 1 volume of isopropanol at -20°C for 1 h. The precipitate was collected by centrifugation and resuspended in 200 ul of 10 mM Tris-HCl (pH 7.2), 1 mM Na_2EDTA and 250 mM NaCl. Subgenomic CaMV DNA species were resolved from full genome length CaMV DNA species by centrifugation in 10%-40% (w/w) linear sucrose density gradients containing 10 mM Tris-HCl (pH 7.2), 1 mM Na_2EDTA and 250 mM NaCl in a Beckman SW-41 rotor for 14 h at 30,000 rpm and 20°C. Fractions (0.4 ml) were collected from the top of the gradient. CaMV DNA was localized in the gradient fractions and further characterized by agarose gel electrophoresis and hybridization to ^{32}P-CaMV DNA after transfer to nitrocellulose. Agarose gel electrophoresis, blotting, DNA-DNA hybridization and nick translations were performed as described previously (9).

Isolation of form I subgenomic CaMV DNA. Deproteinized nuclear lysates or sucrose gradient fractions which contained form I subgenomic CaMV DNA were suspended in 10 mM Tris-HCl (pH 8.0), 1 mM Na_2EDTA and 10 mM NaCl (TES), adjusted to 0.77 g/ml cesium chloride and 300 µg/ml ethidium bromide and subjected to centrifugation at 40,000 rpm for 48 h and 20 C in a Beckman Ti-75 rotor. The gradient was fractionated and CaMV DNA was localized in the gradient fractions as described above.

Electron microscopy. Subgenomic CaMV DNA was either relaxed with

topoisomerase I as described below or directly prepared for electron microscopy by the aqueous cytochrome C spreading method (29). DNA was visualized with an Hitachi H600 electron microscope and photographed.

Topoisomerase I relaxation of supercoiled DNA. Supercoiled DNA was relaxed with wheat germ topoisomerase I (Promega Biotec, Madison,WI.) as recommended by the supplier and analyzed by agarose gel electrophoresis. When DNA was to be examined by electron microscopy, relaxation was performed in 150 mM ammonium acetate (pH 7.5), 1 mM Na_2EDTA, 1 mM dithiothreitol, 20% glycerol and 100 µg/ml BSA. After topoisomerase incubations, DNA was phenol extracted, precipitated with isopropanol and resuspended in TES.

Genomic and subgenomic CaMV clones. A genomic clone (pMnS-4) of CaMV (isolate CM1841-Mn) was constructed by linearizing CM1841-Mn with Sal I and inserting it into the Sal I site of pBR322. Subgenomic Eco RI clones of CaMV (isolate CM4-184) were prepared by inserting Eco RI digested CM4-184 DNA into the Eco RI site of pBR325 or pBR322. All clones were propagated in E. coli LE392 in accordance with the NIH Guidelines for Recombinant DNA.

RESULTS

When cleared lysates of nuclei isolated from CaMV-infected turnip leaves are subjected to sucrose density gradient centrifugation and the gradient is analyzed for the presence of CaMV DNA, a population of subgenomic CaMV DNA species with sedimentation coefficients less than the genome length CaMV minichromosome are observed (Fig. 2). The subgenomic CaMV DNA species (before deproteinization) have sedimentation coefficients on sucrose density gradients greater than that expected for deproteinized DNAs of comparable size. This indicates that the subgenomic CaMV DNA species may be in the form of minichromosomes or in some form of nucleoprotein complex. Protein blotting experiments indicate that the nucleoprotein complexes do not contain virus coat protein (T.J. Guilfoyle, unpublished). CaMV DNA encapsidated into virions is not observed in Figure 2 because virion DNA is only partially deproteinized by Sarkosyl (9, 11), and the partially deproteinized virion DNA is removed from the aqueous phase by phenol extraction.

When deproteinized DNA isolated from cleared lysates of CaMV-infected turnip leaf nuclei is fractionated on sucrose density gradients and then analyzed by agarose gel electrophoresis, the subgenomic CaMV species are resolved into two populations (Fig. 3). Populations of subgenomic CaMV DNA are represented by the two arcs of hybridization which end at either the genome length form I CaMV DNA or the genome length form II and form III CaMV DNA (Fig. 3). These results suggest that subgenomic CaMV DNA consists of a population of supercoiled

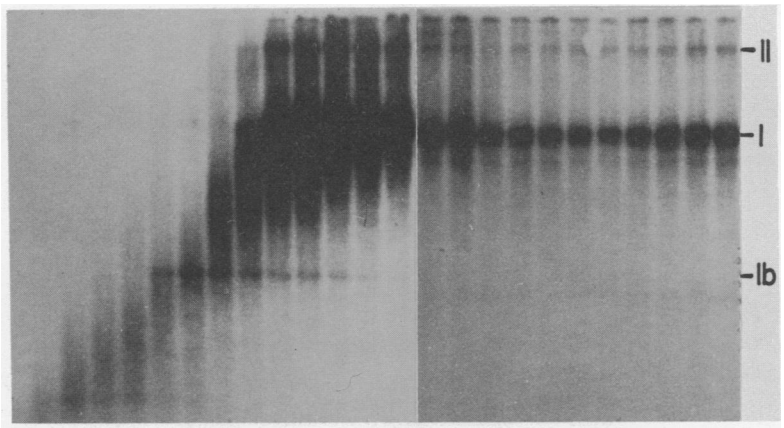

Figure 2. Sucrose density gradient centrifugation of minichromosomes. Cleared lysate, containing minichromosome from CaMV-infected turnip leaf nuclei was subjected to sucrose density gradient centrifugation. The gradient was fractionated, and the DNA in an aliquot of each fraction was deproteinized with Sarkosyl, phenol extracted and precipitated. The DNA was subjected to electrophoresis in an 0.8% agarose gel and transferred to nitrocellulose. ^{32}P-CaMV DNA was used as a probe. The lanes from left to right represent the sucrose gradient fractions from the top to the bottom of the sucrose gradient, respectively. I and II mark the location of genome length form I and II CaMV DNA. Ib marks the location of Ib-DNA.

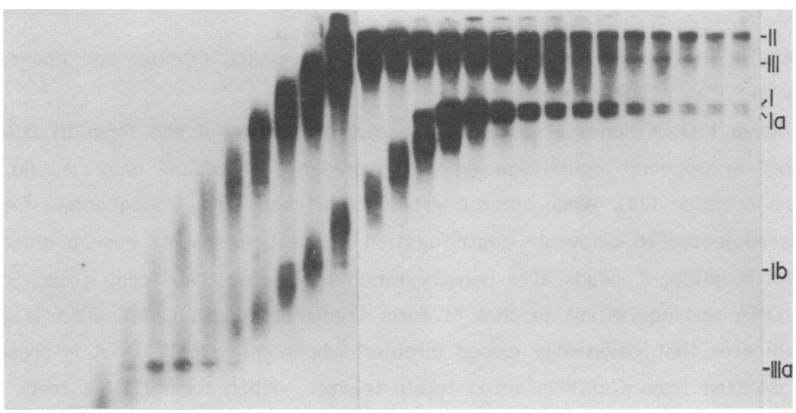

Figure 3. Sucrose density gradient centrifugation of nuclear, cleared lysate DNA. The DNA contained in a nuclear cleared lysate from CaMV-infected tissue was deproteinized and subjected to sucrose density gradient centrifugation. The gradient was fractionated, and the DNA in a portion of each fraction was subjected to 0.8% agarose gel electrophoresis and transferred to nitrocellulose. ^{32}P-CaMV DNA was used as a probe. The lanes from left to right represent fractions from the top to the bottom of the sucrose gradient, respectively. I, II and III mark the location of genome length form I, II and III CaMV DNA, respectively. Ia, Ib and IIIa mark the location of abundant subgenomic CaMV DNA species.

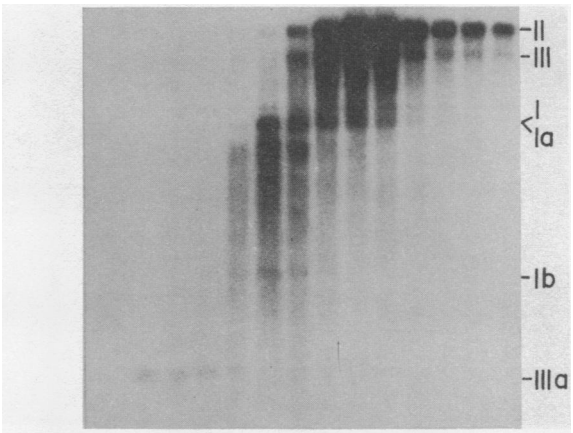

Figure 4. Cesium chloride-ethidium bromide gradient centrifugation of subgenomic form I CaMV DNA. Sucrose density gradient purified form I CaMV DNA was subjected to centrifugation in the presence of cesium chloride and ethidium bromide. The gradient was fractionated and the DNA in an aliquot of each gradient fraction was subjected to electrophoresis in an 0.8% agarose gel. The DNA was then transferred to nitrocellulose and probed with ^{32}P-CaMV DNA. The lanes from left to right represent fractions from the bottom to the top of the gradient, respectively. I, II and III indicate the location of genome length form I, II and III CaMV DNA, respectively. Ia, Ib and IIIa mark the location of abundant subgenomic CaMV DNA species. Hybridization to DNA between Ia and Ib includes the bulk of the supercoiled subgenomic DNAs.

subgenomic CaMV DNA species and a mixture of relaxed circular and linear subgenomic CaMV DNA species.

Form I DNA bands at a density greater than form II and form III DNA when subjected to isopycnic centrifugation in the presence of cesium chloride and ethidium bromide (30). When sucrose gradient-enriched form I subgenomic CaMV DNA is subjected to isopycnic centrifugation in the presence of cesium chloride and ethidium bromide, it bands at a density which is greater than form II and form III CaMV DNA and equivalent to that of form I genome length CaMV DNA (Fig. 4). This indicates that covalently closed circular subgenomic CaMV DNA is present in nuclei isolated from CaMV-infected turnip leaves. When form I DNA from the cesium chloride-ethidium bromide gradient analyzed in Figure 4 is examined by electron microscopy, the majority of the molecules in the size range of 0.5-8 kb appear to be supercoiled (Fig. 5A). When this DNA is treated with wheat germ topoisomerase I, relaxed circular molecules ranging in size from less than 10% of CaMV genome length to nearly full genome length are observed (Fig 5B). Although we have no direct evidence that the molecules shown in Figure 5 are CaMV DNA, two observations support this conclusion. First, nuclei isolated from uninfected

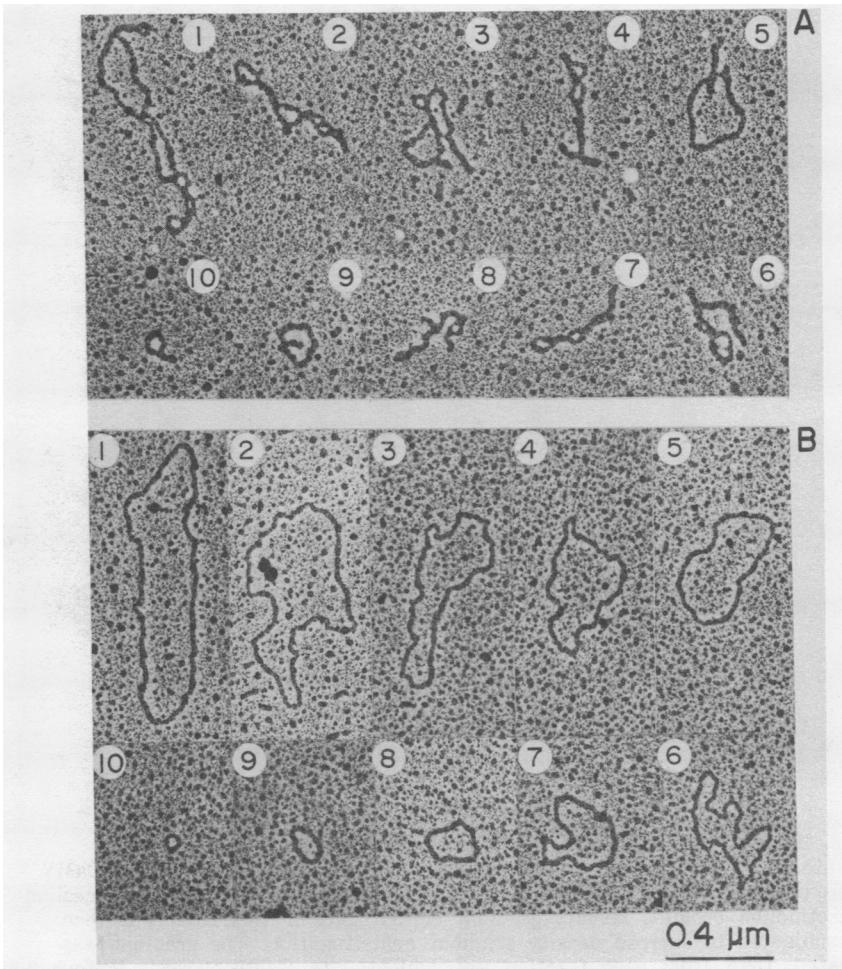

Figure 5. Size range of form I subgenomic CaMV DNA visualized by electron microscopy. Purified subgenomic form I CaMV DNA (A) or topoisomerase I relaxed subgenomic form I CaMV DNA (B) was spread in the presence of cytochrome C and photographed at 50,000 x magnification. CaMV DNA molecules are arranged in order of decreasing size from (1) through (10). DNA used for electron microscopy was purified by sucrose gradient plus cesium chloride-ethidium bromide density gradient centrifugation.

turnip leaves do not contain any supercoiled DNA molecules which band at the same density on cesium chloride-ethidium bromide as form I CaMV DNA (data not shown). Second, when unfractionated DNA from nuclear lysates prepared from CaMV-infected turnip leaves are analyzed by electron microscopy, supercoiled or

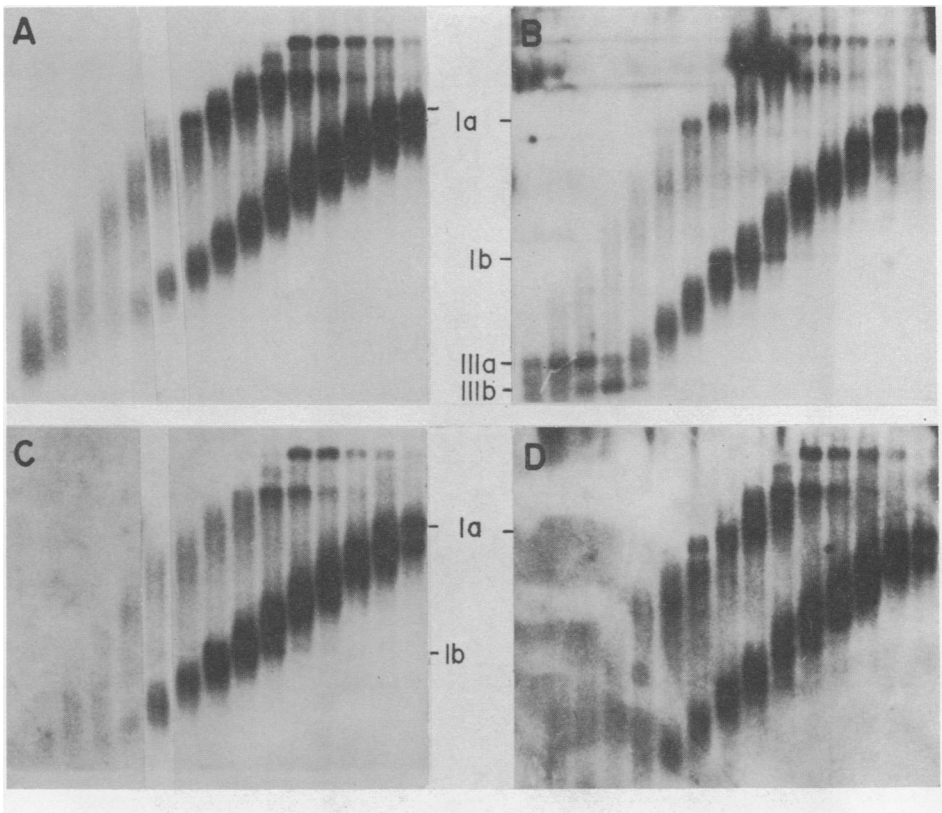

Figure 6. Hybridization analysis of subgenomic form I CaMV DNA. Form I subgenomic CaMV DNA which did not contain full genome length form I CaMV DNA was prepared by sucrose density gradient centrifugation followed by cesium chloride-ethidium bromide density gradient centrifugation. This DNA was then further subjected to sucrose density gradient centrifugation. The gradient was fractionated, DNA from each fraction was subjected to electrophoresis in four 0.8% agarose gels, and the DNA was transferred to nitrocellulose. The filters were probed with nick-translated Eco RI subclones of the CaMV genome: (A) the Eco RI-a fragment, (B) the Eco RI-b fragment, (C) the Eco RI-c fragment and (D) the Eco RI-d fragment (see Fig. 1). Ia, Ib, IIIa and IIIb mark the location of abundant subgenomic CaMV DNA species.

relaxed circular molecules greater than genome length (i.e., 8 kb) CaMV DNA are not observed (data not shown). We consider it unlikely that the 8 kb size of full genome length CaMV DNA and the upper size limit (i.e., 8 kb) of supercoiled and relaxed circular DNA molecules observed by electron microscopy is coincidental.

To determine if portions of the CaMV genome are preferentially deleted to produce the subgenomic species, form I subgenomic CaMV DNA was hybridized to Eco RI subclones of the CaMV genome (Fig. 6; see Fig. 1 for the locations of the

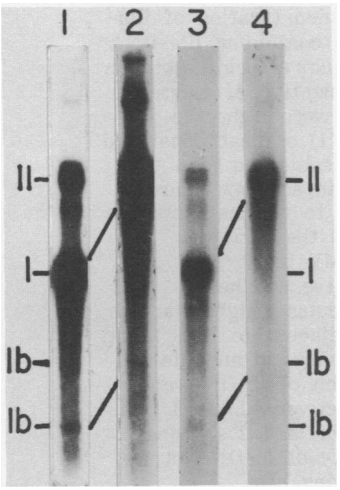

Figure 7. Topoisomerase I relaxation of form I subgenomic CaMV DNA. Form I subgenomic CaMV DNA purified from cleared lysates of CaMV-infected turnip leaf nuclei and subjected to cesium chloride-ethidium bromide centrifugation was incubated in the absence (lane 1 and 3) or presence (lane 2 and 4) of wheat germ topoisomerase I, the DNA was then extracted, subjected to electrophoresis in an 1.0% agarose gel, transferred to nitrocellulose and hybridized with ^{32}P-CaMV DNA. I, Ib and II mark the location of form I genome length CaMV DNA, Ib-DNA and form II genome length CaMV DNA, respectively. Lanes 3 and 4 are lighter exposures of lanes 1 and 2, respectively.

Eco RI fragments in the CaMV genome). All size classes of form I subgenomic CaMV DNA hybridize to each of the CaMV Eco RI subclones. This indicates that specific regions of the CaMV genome are not preferentially deleted to produce the form I subgenomic CaMV DNA species and that the different species of subgenomic CaMV DNA may arise by random deletions in the CaMV genome. It is possible, however, that multiple small specific deletions are occurring during the formation of the different subgenomic CaMV DNA species and that the subgenomic clones used in our experiments are too large to detect them.

The even distribution of hybridization along the arcs which represent the subgenomic CaMV DNA species (Fig. 3 and 6) indicates that many size classes of subgenomic CaMV DNA species accumulate in nuclei. However, certain subgenomic CaMV DNA species do accumulate preferentially in CaMV-infected tissue. These species are indicated by the darker bands of hybridization within the arc of hybridization to the subgenomic CaMV DNA (Fig. 2 and 3; bands labeled Ia, Ib and IIIa). Additional discrete CaMV DNA species less abundant than Ia, Ib, and IIIa are observed on some blots.

Two of these species (Ia-DNA and Ib-DNA) appear to be form I in nature since they band with the form I DNA when subjected to isopycnic centrifugation in the presence of cesium chloride and ethidium bromide (Fig. 4). All of the form I subgenomic CaMV DNA species, including the species Ia-DNA and Ib-DNA, are relaxed by wheat germ topoisomerase I (Fig. 7; supercoiled genome length and Ia-DNA have comigrated on this gel as have the relaxed circular forms of these species). Although we have not definitively demonstrated that form I Ib-DNA

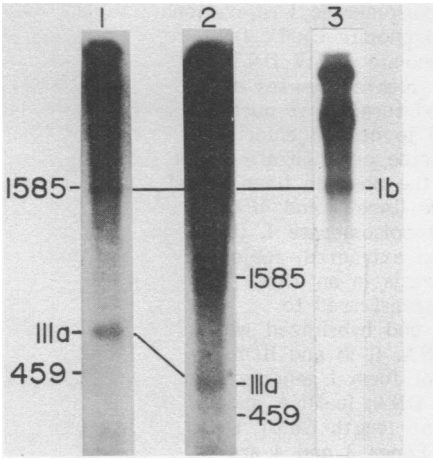

Figure 8. Heat denaturation of IIIa-DNA. Fractions containing IIIa-DNA purified by cesium chloride-ethidium bromide centrifugation of cleared nuclear lysates were either untreated (lane 1) or heat denatured (lane 2 and 3), subjected to electrophoresis in an 1.4% agarose gel, transferred to nitrocellulose, and probed with the Eco RI-b subclone of CaMV DNA. The locations of native and heat denatured molecular weight markers are indicated adjacent to lane 1 and lane 2, respectively. Ib and IIIa mark the location of Ib-DNA and IIIa-DNA, respectively. Lane 3 is a short exposure of lane 2 showing that the mobility of Ib-DNA is unaltered following heat denaturation.

relaxes to the band labeled Ib in Fig. 7 (lanes 2 and 4), it is clear that topoisomerase treatment results in a mobility shift of Ib-DNA. These species are all resistant to heat denaturation (Fig. 8; lanes 1 and 3). This latter result along with results using topoisomerase indicates that these species are covalently closed circles. Ia-DNA is slightly smaller than full genome length CaMV DNA and hybridizes to all of the CaMV Eco RI subclones (Fig. 6). Ib-DNA is about 2 kb and hybridizes only to subclones which contain the CaMV Eco RI-b or Eco RI-c DNA fragments (Fig. 6). A second round of hybridization to blots A-D in Figure 6 using ^{32}P-labeled full genome length CaMV DNA (i.e., plasmid pMnS-4) indicated that bands Ib, IIIa and IIIb were clearly visible on all filters (data not shown) and could not have been obscured in the first round of hybridization (i.e., these bands were not obscured by any intensive hybridization on filters A and D).

The other abundant CaMV DNA species (IIIa-DNA) appears to be a double-stranded linear DNA of about 630 base pairs, which heat denatures to yield a single-stranded DNA of about 630 nucleotides (Fig. 8). This species bands at a density greater than form I DNA when subjected to isopycnic centrifugation in the presence of cesium chloride and ethidium bromide (Fig. 4). The increased density probably results from RNA covalently attached and/or hybridized to CaMV DNA. A portion of this DNA species is reduced in size following treatment with ribonuclease A (L. Marsh and T. J. Guilfoyle, unpublished). This CaMV DNA species hybridizes only to subclones containing the CaMV Eco RI-b fragment. A single-stranded CaMV DNA species (sa-DNA) approximately the same size as IIIa-DNA which has RNA attached and hybridizes to the CaMV Eco RI-b fragment has

been previously observed in tissue extracts and CaMV virus particles (21, 26); however, the relationship of the small DNA species observed here to those described previously (21, 26) is not clear. It is clear, nevertheless, that species IIIa does not represent an abundant covalently closed circular DNA like species Ib. Figure 6B also shows an additional abundant subgenomic CaMV DNA species (IIIb-DNA) which hybridizes only to CaMV subclones containing the Eco RI-b fragment (IIIb-DNA is not observed in other blots (i.e., Fig. 3) because this species migrated off of the agarose gels). The nature of IIIb-DNA has not been investigated further.

DISCUSSION

Nuclei isolated from CaMV-infected turnip leaves contain forms I, II and III subgenomic CaMV DNA species. These subgenomic CaMV DNA species appear to be present as minichromosomes or nucleoprotein complexes. The form II and III subgenomic species probably arise by nicking of the form I species during the DNA isolation procedure. The form I subgenomic species range in size from about 10% of full CaMV genome length to nearly full genome length.

There are two form I subgenomic CaMV DNA species which appear to accumulate preferentially in the nuclei of infected tissue. One of these species (Ia-DNA) is nearly full CaMV genome length. This species is present in amounts nearly equal to the full genome length form I CaMV DNA. In this study, tissue was infected with pMnS-4, a genomic clone of CaMV isolate CM1841-Mn. CaMV is known to undergo a deletion mutation which produces CaMV isolate CM4-184, an infective isolate with a 421 base pair deletion (5,31). The form Ia-DNA species (reported here) is not produced by the deletion event described above, since it contains the Xho 1 restriction site which is deleted in CM4-184 (E. Hack and T.J. Guilfoyle, unpublished observation). However, Ia-DNA may be produced by a small deletion in a different portion of the genome. The other abundant subgenomic form I DNA species (Ib-DNA) is considerably smaller (about 2 kb) than full genome length. This species hybridizes only to clones containing the CaMV Eco RI-b or Eco RI-c fragments, and this indicates that a large portion or all of the CaMV Eco RI-a and Eco RI-d fragments is deleted in this DNA species.

Leaves infected with the naturally occurring CaMV isolate CM1841-Mn (data not shown) or leaves from plants infected with a genomic clone (pMnS-4) were used in this study. In both cases, the infected tissue contained a population of subgenomic CaMV DNA and both populations appeared identical. These results indicate that the subgenomic populations of CaMV DNA arise quickly in infected tissue and do not accumulate as the result of serial propagation of the virus over many plant generations. Purified CaMV virion DNA does not contain this population

of subgenomic DNA, and supercoiled molecules are not encapsidated (11). It is likely that the population of subgenomic CaMV DNA arises anew in each infection event.

It has been proposed that CaMV may replicate by a mechanism similar to retroviruses, employing reverse transcriptase and replicating through an RNA intermediate (21-23). New species of retroviruses containing deletions in their genome arise frequently (32-35). It has been previously shown that when avian sarcoma virus is propagated in a Japanese quail tumor cell line (QT-6), specific subgenomic covalently closed circular DNA species accummulate in the nucleus (35). When retroviruses replicate, reverse transcriptase initiates at a primer near the 5'-end of the replicative transcript and proceeds to the 5'-end. To complete replication, reverse transcriptase must transfer to a homologous region at the 3'-end of this or another replicative transcript (for review see 24). It has been proposed that deletions can occur when reverse transcriptase reaches a break in the replicative transcript and transfers to another homologous region within the genome (36). Subgenomic form I CaMV DNA could arise by a similar mechanism. However, RNA breakage would not be necessary since aberrant transfer could occur when reverse transcriptase reaches the 5'-end of the 35S transcript. The rapid accumulation of subgenomic CaMV DNA species by intragenomic recombination events provides further circumstantial evidence that CaMV may replicate by reverse transcription.

The CaMV genome has the potential to code for eight polypeptides (8,37,38). However, only three major species of poly A RNA (8S, 19S and 35S) and a poly A RNA population of heterogeneous size have been identified in infected tissue (15-20). The 8S RNA and the 35S RNA have a common 5'-end. The 5'-end of the 19S transcript precedes open reading frame VI (18-20). No transcription promoters or RNAs with 5'-ends which precede the other open reading frames have been identified to date. Other open reading frames might be translated after processing of the 35S transcript or translation could occur from a polycistronic mRNA. Another possible mechanism is that intragenomic recombination which produces the subgenomic CaMV DNA could juxtapose one of the two identified transcription promoters adjacent to the other open reading regions facilitating their transcription and translation. On the other hand, the subgenomic CaMV DNA species may represent a dead end product of aberrant CaMV replication.

The Ia-DNA and Ib-DNA species may arise preferentially by the intragenomic recombination mechanism described above; however, since they accumulate they may be capable of replication. This replication, if it occurs, may require the presence of intact virus genomes to act as helpers. CaMV can be used as a vector

to carry foreign DNA into turnip; however, the foreign DNA which can be maintained has a maximum size of 450 bp (39). If Ia-DNA and Ib-DNA can replicate, they may have the potential to act as vectors which can accommodate more foreign DNA than full genome length CaMV DNA.

Nuclei from CaMV-infected tissue also contain a 630 base pair linear double-stranded subgenomic DNA species (IIIa-DNA). IIIa-DNA hybridizes only to subclones containing the CaMV Eco RI-b fragment and has a density on cesium chloride-ethidium bromide gradients which is greater than form I DNA. This molecule may be a double-stranded form of sa-DNA (21,26). IIIa-DNA or a similar species is encapsidated in CaMV virus particles (11). IIIa-DNA may represent the strong stop species seen in reverse transcription, but, in this case, a complementary strand has been synthesized.

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REFERENCES

1. Hull, R. (1979) in Nucleic Acids in Plants, Hall, T.C. and Davies, J.W. Eds., Vol. II, pp. 3-29, CRC Press, Boca Raton.
2. Shepherd, R.J. (1979) Ann. Rev. Plant Physiol. 30, 405-423.
3. Hohn, T., Richards, K. and Lebeurier, G. (1982) Curr. Top. Micro. Immun. 96, 193-236.
4. Howell, S.H. (1982) Ann. Rev Plant Physiol. 33,609-650.
5. Hull, R. and Howell, S.H. (1978) Virology 86,482-493.
6. Volovitch, M., Drugeon, G. and Yot, P. (1978) Nucl. Acids Res. 5, 2913-2925.
7. Richards, K.E., Guilley, H. and Jonard, G. (1981) FEBS Letters 134, 67-70.
8. Franck, A., Guilley, H., Jonard, G., Richards, K. and Hirth, L. (1980) Cell 21, 285-294.
9. Olszewski, N., Hagen, G. and Guilfoyle, T.J. (1982) Cell 29, 395-402.
10. Guilfoyle, T.J., Olszewski, N.E. and Hagen, G. (1983) in Structure and Function of Plant Genomes, Dure, L.S. and Ciferri, O. Eds., Plenum Press, New York, in press.
11. Guilfoyle, T.J., Olszewski, N., Hagen, G., Kuzj, A. and McClure, B. (1983) in UCLA Symposia on Molecular and Cellular Biology, Goldberg, R.B. Ed., New Series Vol XII, Alan R. Liss, New York, in press.
12. Menissier, J., de Murcia, G., Lebeurier, G. and Hirth, L. (1983) EMBO J. 2,1067-1071.
13. Guilfoyle, T.J. (1980) Virology 107, 71-80.
14. Howell, S.H. and Hull, R. (1978) Virology 86, 468-481.
15. Odell, J.T. and Howell, S.H. (1980) Virology 102,349-359.
16. Al Ani, R., Pfeiffer, P., Whitechurch, O., Lesot, A., Lebeurier, G. and Hirth, L. (1980) Ann. Virol. 131E, 33-53.
17. Odell, J.T., Dudley, R.K. and Howell, S.H. (1981) Virology 111, 377-385.
18. Covey, S.N., Lomonossoff, G.P. and Hull, R. (1981) Nucl. Acids Res. 9, 6735-6747.
19. Dudley, R.K., Odell, J.T. and Howell, S.H. (1982) Virology 117, 19-28.
20. Guilley, H., Dudley, R.K., Jonard, G., Balazs, E. and Richards K.E. (1982)

Cell 30, 763-773.
21. Guilley, H., Richards, K.E. and Jonard, G. (1983) EMBO J. 2, 277-282.
22. Hull, R. and Covey, S.N. (1983) Trends Biochem. Sci. 8, 119-121.
23. Pfeiffer, P. and Hohn, T. (1983) Cell 33, 781-789.
24. Varmus, H.E. (1983) in Mobile Genetic Elements, Shapiro, J.A. Ed., pp. 411-503, Academic Press, New York.
25. Summers, J. and Mason, W.S. (1982) Cell 29, 403-415.
26. Covey, S.N., Turner, D. and Mulder, G. (1983) Nucl. Acids Res. 11, 251-264.
27. Hull, R. and Covey, S.N. (1983) Nucl. Acids Res. 11, 1881-1895.
28. Howell, S.H., Walker, L.L. and Dudley, R.K. (1980) Science 208, 1265-1267.
29. Davis, R.W., Simon, M. and Davidson, N. (1971) Methods Enzymol. 21, 413-428.
30. Radloff, R., Bauer, W. and Vinograd, J. (1967) Proc. Nat. Acad. Sci. USA 57, 1514-1521.
31. Howarth, A.J., Gardner, R.C., Messing, J. and Shepherd, R.J. (1981) Virology 112, 678-685.
32. Vogt, P.K. (1971) Virology 46, 939-946.
33. Martin, G.S. and Duesberg, P.H. (1972) Virology 47, 494-497.
34. Shields, A., Witte, O.N., Rothenberg, E. and Baltimore, D. (1978) Cell 14, 601-610.
35. Guntaka, R.V., Richards, O.C., Shank, P.R., Kung, H.J., Davidson, N., Fritsch, E., Bishop, J.M., and Varmus, H.E. (1976) J. Mol. Biol. 106, 337-357.
36. Coffin, J.M. (1979) J. Gen. Virol. 42, 1-26.
37. Gardner, R.C., Howarth, A.J., Hahn, P., Brown-Luedi, M., Shepherd, R.J. and Messing, J. (1981) Nucl. Acids Res. 9, 2871-2887.
38. Balazs, E., Guilley, H., Jonard, G. and Richards, K. (1982) Gene 19, 239-249.
39. Gronenborn, B., Gardner, R.C., Schaefer, S. and Shepherd, R.J. (1981) Nature 294, 773-776.