The size of encapsidated single-stranded DNA determines the multiplicity of *African cassava mosaic virus* particles

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Geminiviruses are small plant viruses with circular single-stranded (ss) DNA encapsidated in twinned (geminate) particles (see Fig. 1, panel B3). They have been divided into three genera on the basis of their genome organization, host range and insect transmission. Members of the genera *Mastrevirus* and *Curtovirus* have monopartite genomes and infect mainly monocotyledonous or dicotyledonous plants, respectively. Members of the genus *Begomovirus* infect dicotyledonous plants, are whitefly transmitted and have monopartite or bipartite genomes (DNA A and B). The coat protein (CP) of bipartite geminiviruses is encoded by the DNA A molecule (reviewed by Frischmuth, 1999).

Transgenic *Nicotiana benthamiana* plants harbouring a defective interfering (DI) DNA of *African cassava mosaic virus* (ACMV) and control plants were inoculated with ACMV. Virus particles were purified from infected plants, separated in sucrose gradients and fractions were analysed by Southern blotting. Transgenic plant-derived virus particles taken from the top fractions of sucrose gradients contained DI DNA, middle fractions contained a mixture of genomic and DI DNA and bottom fractions contained a mixture of multimeric, genomic and DI DNA. Virus particles from selected top, middle and bottom fractions were analysed by electron microscopy. In fractions containing only DI DNA, isometric particles of 18–20 nm were detected. In fractions containing DI DNA as well as genomic size DNA, isometric and geminate particles were found. Fractions containing multimeric size DNA were found to comprise particles consisting of three subunits adjacent to geminate particles. From these data, it is concluded that the size of encapsidated DNA determines the multiplicity of ACMV particles.

The CPs of mastreviruses, curtoviruses and monopartite begomoviruses are essential for systemic movement within the plant (Briddon *et al*., 1989; Boulton *et al*., 1989; Lazarowitz *et al*., 1989). The CP of bipartite begomoviruses is not required for transport within the plant (Stanley & Townsend, 1986; Gardiner *et al*., 1988), but protects viral DNA during transmission and determines vector specificity (Briddon *et al*., 1990; Azzam *et al*., 1994; Höfer *et al*., 1997).

In this study, we investigated the requirements to form the unique particle structure of geminiviruses using *African cassava mosaic virus* (ACMV), a bipartite member of the genus *Begomovirus* (Stanley & Gay, 1983). ACMV was inoculated onto two *Nicotiana benthamiana* plant lines, a non-transformed control line and a transgenic line containing a DI DNA. The production of the DI DNA transgenic *N. benthamiana* plant line and the inoculation methods used are described by Stanley *et al*. (1990). DI DNA transgenic lines showed ameliorated symptoms associated with reduced virus content (Stanley *et al*., 1990).

Virus particles were isolated from approximately 20–30 g of infected leaf material, essentially as described by Sequeira & Harrison (1982), and separated in 10–40% sucrose gradients in 5 mM Tris–HCl, pH 8.0, 2.5 mM EDTA at 150000 g for 3 h at 4 °C. Gradients were fractionated in 500 pl aliquots and 1/20 of each aliquot (fractions 3–16) was separated by agarose gel electrophoresis. ACMV DNA A- and B-specific probes were used for Southern blots as described previously (Frischmuth & Stanley, 1991).

When lots of particle preparations from transgenic plants were hybridized with ACMV DNA A, genomic size ssDNA was first detected in fraction 4 and increased in amounts up to fraction 6 (Fig. 1A, lanes 3–5). When hybridized with DNA B, DI DNA was detected in fractions 3–12, with maximum accumulation occurring in fraction 4 (Fig. 1A, lane 3). DNA molecules with higher molecular mass were detected from fraction 7 onwards (Fig. 1A, lane 7). A similar distribution was detected on lots of particle preparations from control plants with the exception that no DI DNA was detected when hybridized with DNA B (Fig. 2A). In Southern blot analysis of total nucleic acid extracts, the viral ssDNA form of amplified DI DNA was detected in infected transgenic plants (Stanley *et al*., 1990; Frischmuth & Stanley, 1991). Here we demonstrated...
that this ssDNA form of amplified DI DNA is also encapsidated.

Particles isolated from transgenic and control plants were analysed by electron microscopy (EM). Selected fractions of the sucrose gradients were dialysed against phosphate buffer (60 mM NaK phosphate buffer, pH 6.5) and virus particles were either precipitated onto carbon-coated copper grids or bound to the grid via ACMV-specific antibodies (immunosorbent electron microscopy; ISEM), essentially as described by Sequeira & Harrison (1982). For ISEM, grids were incubated with polyclonal rabbit antiserum (1:500 dilution) raised against ACMV particles (von Arnim et al., 1993).

When fraction 3 of the particle preparations from transgenic plants (Fig. 1A, lane 2) was analysed, particles of 18–20 nm in size, almost exclusively spherical, were detected (data not shown). Particles of this size also bound to the ACMV antibody-coated grids, verifying that these particles contained ACMV CP (Fig. 1B, panel B1). In addition to these small spherical particles, geminate particles of normal shape and size were visible in fraction 4 (Fig. 1B, panel B2). The size of these spherical particles corresponds to approximately one half (or one subunit) of a gemini particle (Fig. 1B, panel B3). The EM picture of these small particles does not show the expected icosahedral shape of ACMV particles; they seem to be flattened on one side (Fig. 1B, panel B3). In fraction 4 of particle preparations from control plants, normal gemini particles were exclusively detected (Fig. 2B, panel B1). However, when fractions 10 and 11 were analysed, particles consisting of three subunits were occasionally detected adjacent to normal geminate particles (Fig. 2B, panel B2).

Fig. 1. (A) Southern blot analysis of sucrose gradient fractions of particle preparations from DI DNA transgenic plants. Blots were hybridized with ACMV DNA A- and B-specific probes. Fractions were taken from top to bottom and fractions 2–11 (lanes 1–10) were analysed. Viral ss DI (ssDI-DNA), genomic (ss-gDNA) and multimeric (ss-mDNA) DNA forms are indicated. (B) EM of virus particles (arrows) isolated from fractions 3 (B1) and 4 (B2). Virus particles shown in B2 were bound to the grid by ACMV-specific antibodies (ISEM) and were magnified by the same degree and mounted close together for size comparison (B3). Bar, 100 nm.

Particles containing DI DNA were purified from infected DI DNA transgenic plants. The DI DNA is encapsidated in spherical particles representing one half of a geminate virus particle. The presence of half-size particles in a virus preparation has previously led to suggestions that these are either broken particles or particles containing smaller DNA molecules (Harrison, 1985; MacDowell et al., 1986). In sucrose gradient fractions containing viral DNA of higher molecular mass, ACMV particles with three subunits were detected. Even particles consisting of four subunits have been described previously in the literature (Briddon & Markham, 1995). This implies that the single coat protein of geminiviruses is able to form particles of different shape and that the structure is apparently determined by the size of the encapsidated DNA.

The systemic spread of geminiviruses is size-dependent (Klinkenberg et al., 1989; Stanley et al., 1990) and, as we have shown here, the size of the encapsidated viral DNA also determines the particle structure of ACMV. The size of
Multicomponent ssDNA viruses, the nanoviruses, cause diseases in banana (Harding et al., 1993), faba bean (Katul et al., 1997) or coconut (Rohde et al., 1990). Nanoviruses are isometric plant viruses with circular ssDNA genomes of about 1·0–1·2 kb in length and a virus particle size of 18–20 nm (Harding et al., 1993). As we have isolated geminivirus particles of similar size to nanoviruses from DI DNA transgenic plants and as numerous non-geminivirus molecules have been found previously in geminivirus-infected plants, it might be that the diseases caused by nanoviruses are an accumulation of these geminivirus-associated molecules, which have become independent of the geminivirus for disease production. On the other hand, particles with one, two, three and four subunits were isolated from geminivirus-infected plants. Furthermore, interfamily recombination between nano- and geminiviruses has been suggested from sequence analysis of ageratum yellow vein virus-associated molecules (Saunders & Stanley, 1999). Therefore, geminiviruses might represent recombinant nanoviruses.

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References


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