A monopartite begomovirus-associated DNA \( \beta \) satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection

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DNA \( \beta \) is a circular single-stranded satellite DNA which co-infects with certain monopartite helper begomoviruses to cause economically important diseases, such as cotton leaf curl disease (CLCuD). DNA \( \beta \) encodes a single protein, \( \beta \)C1. Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus in which both DNA A and DNA B are required for systemic infection. Inoculation of tomato plants with ToLCNDV DNA A alone induced local but not systemic infection, whereas co-inoculation with DNA A and the DNA \( \beta \) associated with CLCuD resulted in systemic infection. DNA \( \beta \) containing a disrupted \( \beta \)C1 open reading frame (ORF) did not mobilize DNA A systemically. Co-inoculation of plants with DNA A and a construct of the \( \beta \)C1 ORF, under the control of the cauliflower mosaic virus 35S promoter, resulted in the systemic movement of DNA A. In inoculated tobacco and onion epidermal cells, \( \beta \)C1 fused to GFP was localized at the cell periphery in association with punctate bodies, around and within the cell nucleus and with the endoplasmic reticulum. It is concluded that heterologous \( \beta \)C1 protein can replace the movement function of the DNA B of a bipartite begomovirus. Evidence is also provided that tomato leaf curl virus-encoded C4 protein confers the same movement function to ToLCNDV DNA A. The intracellular distribution of \( \beta \)C1 is consistent with the hypothesis that it has a role in transporting the DNA A from the nuclear site of replication to the plasmodesmatal exit sites of the infected cell.

INTRODUCTION

Geminiviruses (family Geminiviridae) have circular single-stranded DNA genomes encapsidated in twinned quasi-isometric particles and are responsible for major crop losses worldwide (Moffat, 1999). The largest genus, Begomovirus, comprises viruses transmitted by the whitefly Bemisia tabaci. Most begomoviruses have bipartite genomes, termed DNA A and DNA B. The DNA A component encodes proteins required for viral DNA replication and encapsidation, whereas DNA B encodes two proteins that are essential for systemic movement. A small number of begomoviruses have a monopartite DNA genome that resembles the DNA A of bipartite begomoviruses. This DNA carries all gene functions for replication and pathogenesis (Stanley et al., 2005).

Small circular single-stranded DNA satellites containing a single open reading frame (ORF), termed DNA \( \beta \), have been found associated with certain monopartite begomovirus infections. Since they were first reported in 2000, over one hundred full-length DNA \( \beta \)s have been cloned and sequenced (Mansoor et al., 2006). They consist of approximately 1350 nt and depend on their helper begomoviruses for replication and encapsidation. DNA \( \beta \) contributes to the production of symptoms and enhanced helper virus accumulation in certain hosts. The process through which DNA \( \beta \) regulates pathogenesis is unknown, but it might affect the replication of its helper virus by either facilitating its spread in host plants, or by suppressing host gene silencing (Saunders et al., 2000). The DNA \( \beta \)-encoded protein, \( \beta \)C1, is the determinant of both pathogenicity and suppression of gene silencing (reviewed by Briddon & Stanley, 2006).

Depending on the particular begomovirus, five different proteins are involved in mediating virus transport through host plants: the coat protein (CP), the nuclear shuttle protein (NSP), the movement protein (MP), the C4 protein and the V1 protein (Rojas et al., 2005). Unlike monopartite begomoviruses, the CP of bipartite begomoviruses is not required for cell-to-cell or long-distance movement. For bipartite begomoviruses, cell-to-cell movement function is carried out by two proteins encoded by
the DNA B component, NSP and MP. In the case of monopartite begomoviruses, two proteins, V1 and C4, have been implicated in cell-to-cell movement function (Jupin et al., 1994; Rojas et al., 2001).

We have demonstrated previously that the DNA β associated with the cotton leaf curl disease (CLCuD) encodes the pathogenicity protein, termed βC1, since transgenic Nicotiana tabacum plants expressing either the βC1 gene or a dimeric DNA β construct produced virus-like leaf curl symptoms (Saeed et al., 2005). We report here that DNA β can replace the movement function of the DNA B of a bipartite begomovirus, thus showing that it has a role in virus movement.

**METHODS**

**Infectious clones and transient expression constructs.** Infectious clones of the DNA β component with CLCuD, and the DNA A and DNA B of tomato leaf curl New Delhi virus (ToLCNDV) were prepared as described previously (Padidam et al., 1995; Briddon et al., 2001), as were transient expression constructs of the βC1 gene (Saeed et al., 2005) and the tomato leaf curl virus (TLCV)-encoded C4 gene under the transcriptional control of the 35S cauliflower mosaic virus (CaMV) promoter (Krake et al., 1998).

**Inoculation of plants and analysis of viral DNA.** Graft transmission was done by top grafting (Crete et al., 2001). Inoculation of tomato plants by biolistic bombardment was carried out using partial tandem dimers of ToLCNDV DNA A in pBluescript SK (--) and DNA B in pgem-7zf (+) (Padidam et al., 1995), and a tandem dimer of DNA β in pBluescript SK (+), 35S:βC1 in pART7 (Saeed et al., 2005), and 35S:TLCV C4 in pIT163 (Krake et al., 1998). DNA was deposited onto gold particles and used for inoculation at a rate of 0.5 μg DNA and 250 μg gold particles per plant (Selth et al., 2005). After inoculation, the plants were grown at 25–27°C under artificial light (150 μE·s⁻¹·m⁻²) with a 16 h photoperiod.

Viral DNAs were detected by dot blot or Southern blot analysis using 32P-labelled DNA probes to DNA A (EcoRV and PstI fragment, nt 307–2113), DNA B (SphI and PstI fragment, nt 370–2068), and DNA β (PstI monomer). The probes were prepared as previously described (Drey et al., 1993). Plants inoculated with DNA A alone were tested by PCR using DNA A specific primers (798V, 5′-AACACTGAGATGACACCGAGCAGGTA-3′ and 901R, 5′-CATCAAGTCTTA-3′) to amplify DNA A could be detected in the newly developing leaves (Fig. 2a; lane 1), but no DNA B of ToLCNDV developed downward leaf curling 6–7 days post-inoculation (d.p.i.) and by 21 d.p.i. the infected plants showed pronounced reduction in height and leaf size. In contrast, tomato plants inoculated with DNA A alone remained symptomless (Fig. 1a, b; Table 1). Plants inoculated with DNA A alone lacked detectable levels of DNA A in the distal leaves when tested by dot blot hybridization at 13–16 d.p.i. (data not shown). Twelve of the DNA A-inoculated plants were further analysed by Southern blotting. The inoculated leaves accumulated replicative forms of DNA A (Fig. 2a, lane 1), but no DNA A could be detected in the newly developing leaves (Fig. 2a, lane 2). PCR did not detect DNA A in extracts of distal leaves, whereas amplicons of the expected size were obtained from inoculated leaves (results not shown). Thus, ToLCNDV DNA A alone was able to replicate in inoculated leaves, but did not spread systemically. To test whether a DNA β satellite could influence the pattern of DNA A infection, tomato plants were inoculated with a mixture of DNA A and the DNA β associated with CLCuD (Briddon et al., 2001). A proportion of tomato plants showed systemic leaf curling at 13–16 d.p.i. and by 28 d.p.i. had developed pronounced leaf curling, stunting, vein thickening and enations (Fig. 1c, Table 1). Dot blot analysis detected both DNA A and DNA β in the new symptomatic leaves of 11 plants, whereas new leaves of non-symptomatic plants lacked detectable levels of either DNA (data not shown). Southern blot hybridization at 13–16 d.p.i. confirmed the presence of replicative forms of both DNA A and DNA β in the distal leaves of these plants.
Fig. 2a, c, lanes 5 and 6). However, these plants accumulated lower levels of viral DNA than plants inoculated with DNA A and DNA B (Fig. 2a, lanes 3–6). These results indicated that the DNA β-encoded protein can mobilize ToLCNDV DNA A from sites of inoculation to the distal tissues, presumably by providing the movement function normally provided by DNA B.

The DNA β associated with CLCuD encodes a pathogenicity protein, βC1 (Saeed et al., 2005). To determine whether this protein was responsible for the spread of DNA A from the site of inoculation, tomato plants were co-inoculated with constructs of DNA A and a DNA β mutant that had two stop codons introduced in the βC1 ORF (Saeed et al., 2005). Both DNA A and the mutant DNA β accumulated in the inoculated leaves of the 12 plants (Fig. 2a,c, lane 7). However, no systemic symptoms were observed in any of the inoculated plants (Fig. 1d, Table 1), and none of these plants accumulated a detectable level of either DNA A or mutant DNA β in systemic leaves when tested by Southern blot hybridization (Fig. 2a, c, lane 8). These results indicate that the ability of DNA β to mediate systemic spread of DNA A was dependent on βC1 expression.

**Table 1.** Infectivity in tomato plants of tomato leaf curl New Delhi virus DNA A in combination with DNA β, p35S:βC1 and p35S:TLAV-C4 expression constructs

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Infectivity (infected*/inoculated)</th>
<th>Symptom type</th>
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<tr>
<td></td>
<td>Experiment</td>
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<tr>
<td>DNA A</td>
<td>0/30</td>
<td>0/30</td>
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<tr>
<td>DNA A + DNA B</td>
<td>14/14</td>
<td>3/3</td>
</tr>
<tr>
<td>DNA A + DNA β</td>
<td>5/18</td>
<td>4/16</td>
</tr>
<tr>
<td>DNA A + DNA β Mut</td>
<td>0/20</td>
<td>0/15</td>
</tr>
<tr>
<td>DNA A + 35S:βC1</td>
<td>2/18</td>
<td>3/16</td>
</tr>
<tr>
<td>DNA A + 35S:βC1 Mut</td>
<td>0/18</td>
<td>0/16</td>
</tr>
<tr>
<td>DNA A + 35S:C4</td>
<td>2/18</td>
<td>1/16</td>
</tr>
<tr>
<td>DNA A + 35S:C4 Mut</td>
<td>0/18</td>
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*Infected plants identified by dot blot hybridization using a ToLCNDV DNA A-specific probe.

**Transcript expression of the βC1 gene enhances local accumulation of ToLCNDV DNA A and permits its systemic movement**

It has been shown previously that co-inoculation with tomato golden mosaic virus (TGMV) DNA A and transient expression constructs of the movement proteins BC1 and BV1 encoded by TGMV DNA B, under the control of the CaMV 35S promoter, enhances local accumulation of TGMV DNA A and permits its systemic spread (Jeffrey et al., 1996). When tomato plants were co-inoculated with ToLCNDV DNA A and the transient expression construct 35S:βC1, a proportion showed mild leaf curling symptoms at 13–16 d.p.i. (Fig. 3c, Table 1). Control tomato plants inoculated with either DNA A alone or with a 35S:βC1 Mut construct developed no symptoms (Fig. 3b).

To test whether transient expression of the βC1 gene could influence the level of accumulation of ToLCNDV DNA A at the site of inoculation, total nucleic acids from tomato leaves which had been co-inoculated with DNA A and the 35S:βC1 construct were assayed at 13–16 d.p.i. A noticeably higher level of DNA A was observed when it was co-inoculated with the 35S:βC1 construct (Fig. 4a, lanes 3 and 4).
than in leaves inoculated with the DNA A alone, or DNA A with the 35S:βC1Mut construct (Fig. 4a, lanes 1 and 2 and lanes 5 and 6). This suggests that localized expression of the βC1 gene increases local accumulation of ToLCNDV DNA A in a manner analogous to that which occurs with DNA B in the TGMV system.

To test whether transient expression of the βC1 gene at the site of inoculation could also mediate the systemic movement of ToLCNDV DNA A, newly developed leaves of the inoculated plants were assayed at 13–16 d.p.i. DNA A was shown to accumulate in symptomatic systemic leaves of tomato plants that had been co-inoculated with DNA A and the 35S:βC1 construct (Fig. 4b, lanes 3 and 4), whereas systemic leaves of non-symptomatic plants lacked detectable levels of DNA A (Fig. 4b, lanes 5 and 6). The level of DNA A accumulation in these plants was similar to the level observed in the plants infected with DNA A and DNA β (Fig. 4b, lanes 3 and 4 and lanes 11 and 12). These results were reproduced in three independent experiments and there was no evidence for the presence of either DNA B or DNA β in these plants when analysed by Southern hybridization (results not shown). Localized expression of βC1 therefore mediates systemic spread of ToLCNDV DNA A.

**Transient expression of the TLCV C4 gene also enhances local accumulation of ToLCNDV DNA A and permits its systemic movement**

The AC4/C4 protein of 11–19 kDa encoded by begomoviruses has some functional similarities to the βC1 protein, particularly in pathogenicity determination and as a suppressor of host gene silencing (Cui et al., 2005, Gopal et al., 2007, Vanitharani et al., 2005). In contrast, the ToLCNDV DNA A encodes a significantly smaller AC4 protein with a Mr of 6.7 (Padidam et al., 1995). TLCV is a monopartite begomovirus that requires a C4 ORF, the positional homologue of AC4, for wild-type symptoms (Rigden et al., 1994). To investigate whether a functional C4 protein might complement missing DNA B functions, the role of TLCV C4 gene in mediating cell-to-cell

![Fig. 2. Detection of viral DNAs in tomato plants. The blots were hybridized with DNA A- (a), DNA B- (b) or DNA β-specific probes (c). The positions of single-stranded (SS), super-coiled (SC), and open-circular (OC) DNA forms are indicated. Each lane was loaded with 5 µg DNA. Ethidium bromide-stained DNA is shown below each lane as loading control. IL, inoculated leaves; Sys, systemically infected leaves.](image)

![Fig. 3. Infectivity of DNA A with either 35S:βC1 or 35S:βC4 construct in tomato plants. Non-symptomatic tomato plants inoculated with DNA A alone (b), compared to plants showing mild symptoms induced by co-infection of DNA A with either 35S:βC1 (c) or 35S:βC4 (d). A control plant inoculated with DNA A and DNA β (e) and a healthy control (a) are shown for comparison. Pictures were taken at 35 d.p.i.](image)
movement of TLCNDV DNA A was also investigated in further experiments. We tested whether the localized expression of the TLCV C4 gene could enhance local accumulation of ToLCNDV DNA A and mediate its systemic spread using the transient expression system described above. Tomato plants were inoculated with either DNA A alone or together with a transient expression construct of C4. A small proportion of tomato plants inoculated with the infectious construct of DNA A together with the 35S: C4 construct showed mild leaf curling symptoms at 13–16 d.p.i. (Fig. 3d, Table 1). In contrast, none of the control plants inoculated with DNA A and the 35S: C4Mut construct developed symptoms.

To determine whether localized expression of the TLCV C4 gene could influence the accumulation of ToLCNDV DNA A in inoculated leaves, leaves of tomato plants that were inoculated with either DNA A alone or together with the 35S: C4 construct were analysed by Southern blotting at 13–16 d.p.i. A noticeably higher level of DNA A was observed when it was co-inoculated with the 35S: C4 construct (Fig. 4a, lanes 7 and 8) than in leaves inoculated with the DNA A alone, or DNA A with the 35S: C4Mut construct (Fig. 4a, lanes 1 and 2 and lanes 9 and 10). These results show that localized expression of the C4 gene enhances the accumulation of ToLCNDV DNA A in inoculated leaves.

To investigate whether transient expression of the C4 gene at the site of inoculation could also mediate the systemic movement of ToLCNDV DNA A to the new leaves, at 13–16 d.p.i. newly developed leaves of the inoculated plants were tested by Southern blot analysis. DNA A was detected in newly developing leaves of the symptomatic tomato plants that were inoculated with DNA A and the 35S: C4 construct (Fig. 4b, lanes 7 and 8), whereas non-symptomatic plants lacked detectable levels of DNA A (Fig. 4b, lane 9, 10). The level of systemic DNA A accumulation in these plants (Fig. 4b, lanes 3 and 4) was similar to the levels observed in plants co-infected with DNA A and DNA β (Fig. 4b, lanes 3 and 4 and lanes 11 and 12). These results show that transient expression of the C4 protein can lead to systemic movement of DNA A, as was shown with βC1.

Wild-type ToLCNDV only requires movement functions at the site of inoculation for systemic infection

We were interested to know whether tomato plants in which DNA A was mobilized from sites of inoculation by transient expression of βC1 or the C4 ORF could sustain systemic infection. Dot blot analyses of symptomatic plants co-inoculated with DNA A and either 35S: βC1 or 35S: C4 showed that DNA A accumulation in these plants at 60 d.p.i. was similar to the DNA A accumulation at 13–16 d.p.i. (results not shown). No βC1 or C4 transcripts were detected in distal tissues by RT-PCR, whereas control plants inoculated with DNA A and DNA β contained the approximately 375 bp product expected from a βC1 transcript (results not shown). Sequence analysis of the DNA A from systemically infected leaves showed that it was identical to that used as the inoculum, and therefore that it had not mutated or recombined with the 35S: βC1 or the 35S: C4 construct.

When scions from these tomato plants were tip-grafted onto healthy tomato plants, mild leaf curling was observed on regrowth from the rootstocks 15–18 days later. Subsequent removal of the scions did not affect the continuing symptom development on the stocks, which remained symptomatic in the glasshouse for up to 1 year, and DNA A was retained throughout this period (data not shown). These results indicate that once ToLCNDV DNA A has spread systemically, it provides the gene functions required for infection.

βC1 protein accumulates at the cell periphery, around and inside the nucleus, and co-localizes with the endoplasmic reticulum

The βC1 protein encoded by the DNA satellite associated with tomato yellow leaf curl China virus (TYLCCNV) has been reported to localize to the cell nucleus (Cui et al., 2005). To test whether the CLCuD βC1 protein also targets the cell nucleus, a construct was synthesized with a translational fusion of βC1 to GFP downstream of the CaMV 35S promoter. The construct was delivered by
biolistic inoculation to tobacco and onion epidermal cells and transient expression was analysed by confocal microscopy. The free GFP control showed fluorescence in both the cytoplasm and the nucleus of inoculated cells (Fig. 5a, h). In contrast, the βC1–GFP fusion protein was seen at the cell periphery, and both around and inside the nucleus (Fig. 5b, i). In some cells (approx. 20%), βC1–GFP was associated with punctate bodies (Fig. 5c, j). We also used the Arabidopsis histone 2B–YFP (Boisnard-Lorig et al., 2001) as a control for nuclear localization, and found the fusion protein exclusively in the nucleus (Fig. 5d, k).

When onion epidermal cells expressing βC1–GFP were plasmolysed to separate the plasma membranes from the cell wall, the fluorescence remained associated exclusively with the detached plasma membrane (Fig. 5l, m). When tobacco and onion epidermis cells expressing βC1–GFP were stained with rhodamine, GFP expression (Fig. 5e, n) coincided with rhodamine fluorescence (Fig. 5f, o), indicating that βC1 co-localized with the endoplasmic reticulum (Fig. 5g, p).

**DISCUSSION**

A DNA β satellite associated with Ageratum yellow vein virus has been shown to substitute for the DNA B of Sri Lankan cassava mosaic virus (SLCMV) to allow infection (Saunders et al., 2002). However, SLCMV DNA A alone can cause systemic infection. Saunders et al. (2002) therefore proposed that SLCMV DNA A has biological characteristics of a monopartite begomovirus, and that the virus probably acquired a DNA B component from Indian cassava mosaic virus. The present study reports that a
satellite DNA $\beta$ from CLCuD can substitute for the movement function of the DNA B of a bipartite begomovirus. DNA $\beta$ containing a disrupted $\beta$C1 ORF did not mobilize the DNA A for systemic infection, indicating that it was the $\beta$C1 protein that was required for movement.

Using biolistic inoculation, it was shown that DNA A components of bean dwarf mosaic virus and *Abutilon* mosaic virus were delivered only to the outer epidermal and cortical layers of bean hypocotyl tissues, and that their further movement to the vascular tissue required DNA B-encoded gene functions (Levy & Czosnek, 2003; Gilbertson et al., 2003; Seo et al., 2004; Sudarshana et al., 1998; Wang et al., 1999). It is therefore likely that ToLCNDV DNA A might have also been delivered to the epidermal tissues where it could replicate, but was unable to spread to the neighbouring cells (Figs 2a and 4a). The localized expression of either the $\beta$C1 or C4 protein may have provided a cell-to-cell movement function that led to the spread of DNA A to the vascular tissues. This was supported by Southern blotting results, where noticeably higher levels of DNA A accumulated in the inoculated leaves when it was co-inoculated with either the 35S : $\beta$C1 or the 35S : C4 construct than in leaves inoculated with DNA A alone, or DNA A with either the 35S : $\beta$C1Mut or the 35S : C4Mut construct (Fig. 4a). This increased accumulation of viral DNA at sites of inoculation is considered to be dependent on cell-to-cell movement (Jeffrey et al., 1996). Hence, it may be inferred that $\beta$C1 protein encodes a cell-to-cell movement function. However, further studies are required to understand the precise role of $\beta$C1 in cell-to-cell movement.

In addition to its role in pathogenicity (Cui et al., 2004; Saeed et al., 2005; Saunders et al., 2004) and virus movement (this study), $\beta$C1 has been reported to be a suppressor of gene silencing (Cui et al., 2005; Gopal et al., 2007). As yet, there is no direct evidence for the involvement of the TLCV-encoded C4 protein in the suppression of gene silencing. However, the ACA4/C4 protein homologues, encoded by African cassava mosaic virus, bhendi yellow vein mosaic virus (BYYMV) and SLCMV, have been identified as suppressors of gene silencing (Gopal et al., 2007; Vanitharani et al., 2005). Many of the plant virus-encoded suppressors of silencing were initially identified as pathogenicity determinants involved in systemic invasion of host plants (Scholthof, 2005). It is likely that silencing suppression activity of $\beta$C1 and C4 proteins could be acting only by blocking a systemic silencing signal. This could explain why $\beta$C1 and C4 proteins are not necessary once the virus reaches the phloem. In a normal situation, without $\beta$C1 or C4, primary silencing signals would be produced in the inoculated leaf, migrate from cell to cell and then be amplified by RDR6-like proteins in the systemic cells. The amplification of the secondary silencing signals in the systemic leaves would therefore prevent viral replication in those areas. $\beta$C1 and C4 could act by preventing or reducing the production of a primary systemic silencing signal in the inoculated leaf and consequently reducing the production of secondary silencing signal in the systemic leaves. With reduced primary silencing signals, the viral DNA would be able to reach a higher concentration in the inoculated leaf. Also, enough time would remain to establish a systemic infection prior to signal amplification, and the need for $\beta$C1 or C4 proteins would be obviated. In agreement with that hypothesis, it has already been documented that *Nicotiana benthamiana* plants impaired in the reception of primary silencing signals are more vulnerable to viral meristem invasion than wild-type plants (Schwach et al., 2005). However, further studies are required to elucidate the precise role of suppression of host defence by the $\beta$C1 or the C4 protein in the systemic infection of ToLCNDV DNA A.

$\beta$C1 fused to GFP localized at the cell periphery, around and inside the nucleus of tobacco and onion epidermis cells. It is unclear whether the accumulation in the nucleus is due to the size of the fusion protein (approx. 43 kDa), which is small enough to diffuse passively through the large nuclear pore complex (Gafni et al., 2002), or is related to some biological role in infection. Recently, Kumar et al. (2006) have reported that the $\beta$C1 encoded by DNA $\beta$ associated with bhendi yellow vein mosaic disease (BYMVD) is localized towards the periphery of the cell. Together, these patterns of localization are similar to that of the tomato yellow leaf curl virus- and maize streak virus-encoded V1 protein, known to mediate cell-to-cell movement (Kotlizky et al., 2000; Rojas et al., 2001). However, Cui et al. (2005) found that $\beta$C1 encoded by DNA $\beta$ associated with TYLCCNV is targeted largely to the cell nucleus. In this instance, $\beta$C1 has a nuclear localization signal (NLS), $45^{\text{PALAKK}}51^\text{},$ and mutation of the NLS resulted in the loss of nuclear localization (Cui et al., 2005). CLCuD and BYMVD $\beta$C1 proteins lack this sequence, providing a possible explanation for their subcellular localization.

Viruses move through infected plants in two steps, cell-to-cell movement via plasmodesmata and long-distance movement through the phloem. Plant viruses use two principal strategies for cell-to-cell movement. One involves binding of movement protein or movement protein complexes with the viral genome, which is either RNA or DNA, and increasing the size exclusion limit of plasmodesmata. The other is dependent on tubule formation (Lucas, 2006). The results obtained using the transient movement assay (Jeffrey et al., 1996) indicated that both $\beta$C1 and TLCV C4 proteins had a role in cell-to-cell movement function. In the present study, co-localization of the $\beta$C1 with endoplasmic reticulum would be consistent with it having a role in intracellular transport from the nucleus to the cell periphery. Recently, Kumar et al. (2006) showed the interaction of BYMVD $\beta$C1 protein with CP using yeast two-hybrid analysis. It is therefore likely that the interaction of CP with $\beta$C1 protein might be involved in the cell-to-cell movement of virus,
analogous to the cooperative interaction of NSP and MP of bipartite begomoviruses (Gafni et al., 2002).

Apart from understanding the role of DNA β in viral movement, this study has both epidemiological and pathological implications. Despite their recent discovery, more than 130 DNA β satellite sequences have now been deposited in GenBank (Rojas et al., 2005). They are associated with monopartite begomoviruses in a wide variety of vegetable and fibre crops, ornamental plants and weeds, mainly throughout Asia and some in Africa (Mansoor et al., 2006). The DNA β associated with CLCuD is responsible for symptom expression of a devastating disease in Pakistan (Mansoor et al., 2006). Given the presence of a large number of begomoviruses throughout Asia and Africa, the promiscuity of DNA β for helper viruses and the ability of DNA β to substitute for DNA B, the chance exists that new diseases such as cotton leaf curl may emerge from bipartite begomoviruses in the form of a monopartite or bipartite begomovirus associated with a DNA β satellite. As suggested by Saunders et al. (2002), monopartite and bipartite begomovirus do not seem to be separated by a rigid boundary.

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