Tissue targeting signals of

Tomato leaf curl virus

by

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Abstract

Tomato leaf curl virus (TLCV; family *Geminiviridae*, genus *Begomovirus*) causes a severe viral disease of cultivated tomato in northern Australia. Geminiviruses are economically-important plant pathogens and possess either one or two circular, single-stranded DNA (ssDNA) genomes each of 2.5-3 kb. Geminiviruses have been reported to replicate in, and localise to, the nuclei of host plant cells. The tissue and intracellular distribution of the monopartite TLCV was investigated by *in situ* hybridization. Contrary to the previous understanding of geminiviral localization, single-stranded (ss) DNA of TLCV accumulated in the cytoplasm. TLCV ssDNA was also found in the nucleus, as were lower levels of replicative form double-stranded (ds) DNA. Under the same conditions, *Tomato golden mosaic virus* (TGMV) ssDNA and dsDNA were found in nuclei. ssDNA of TLCV, TGMV and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) was detected in some xylem vessels under specific hybridization conditions. Phloem specificity of TLCV was partially released by co-infection with TGMV.

To understand the mechanism of TLCV movement, infectious constructs containing a disrupted V1, V2 or C4 ORF were analysed by their localization at the cellular level. The V1 and C4 mutants were able to spread systemically, but less efficiently than wild-type virus. Transgenic plants expressing C4 complemented the C4 mutant virus, allowing it to spread systemically at the same rate as the wild-type virus. The V2 mutant was unable to infect plants systemically, but when bombarded onto a detached leaf was found to replicate in target cells without infecting the neighbouring cells. To characterize the functions of V1, V2 and C4 in TLCV movement, each ORF was

fused to the green fluorescent protein (GFP) and their subcellular localisation was analysed. V1:GFP exhibited a nuclear and cell peripheral localisation, and was probably associated with the endoplasmic reticulum. V2:GFP was targeted to nuclei and accumulated in nucleoli. C4:GFP was observed at the cell periphery in a punctate pattern consistent with plasmodesmatal (Pd) localisation. Plasmolysis of the transiently expressing V1:GFP and C4:GFP cells suggested an association of these viral proteins with Pd. *N. benthamiana* epidermal cells transiently expressing GFP:V1 and GFP:C4 when treated with Brefeldin A (BFA) showed disrupted distribution of GFP suggesting that V1 and C4 proteins were targeted to the cellular periphery via the Golgi apparatus. Using an inducible system it was found that localization of C4:GFP to cellular membrane was rapid.

To understand the role of a host factor in virus movement, subcellular localization of SlUPTG1:GFP fusion protein was studied. SlUPTG1 is a protein closely related to a family of plant reversibly glycosylated peptides and recently in our laboratory it has been shown that it interacts with V1. SlUPTG subcellular localization resembled the localization of V1:GFP where it was mainly associated with cell wall components. Furthermore, the expression of *SlUPTG1* in a transient TLCV replication assay increased the accumulation of viral DNA, suggesting that this host factor plays a role in viral infection. Together, these results have shown that three viral proteins (CP, V1 and C4) are involved in cell-to-cell and long distance movement, and that a host factor has a possible role in viral movement.

RNA silencing is a sequence-specific mechanism regulating gene expression and has been used successfully for antiviral defense against RNA viruses. Similar strategies to develop resistance against DNA containing TLCV and some other geminiviruses

have been unsuccessful. To determine the fate of a transgene carrying homology to the virus, transgenic plants carrying the TLCV *C4* gene, which induces a distinct phenotype, were used. Upon TLCV infection, the phenotype was abolished and the *C4* transcript disappeared. Concurrently, TLCV specific siRNAs were produced. *In situ* hybridization showed abundant levels of TLCV DNA in phloem cells of TLCV-infected *C4* transgenic plants. However, the *C4* transcripts were no longer detectable in non-vascular cells. Analysis of the transgene by methylation sequencing revealed a high level of *de novo* methylation of asymmetric cytosines in both the *C4* ORF and its 35S promoter. A high level of methylation was also found at both symmetric and asymmetric cytosines of the complementary-sense strand of TLCV double-stranded DNA. Given the previous finding that methylated geminiviral DNA is not competent for replication, a model whereby TLCV evades host defence through a population of *de novo* synthesized unmethylated DNA is proposed.

Satellite ßDNA molecules are becoming an emerging threat to global agriculture by their association with an increasing number of begomoviruses. Tissue specificity of a satellite DNA ß molecule associated with *Cotton leaf curl virus* (CLCuV) was identified. Both DNA ß and CLCuV were found to be phloem specific. Furthermore, it was found that DNA ß transreplicates with TLCV in phloem tissues. However, in contrast DNA ß did not transreplicate with TGMV, which is a mesophyll invasive virus.

This work has provided a new insight into the biology of TLCV showing a distinct pattern of localization and mechanism of movement compared with a bipartite begomovirus. Furthermore, the spread of silencing to non-infected tissues upon TLCV infection and escape of TLCV from silencing explains why transgenic resistance is

inconsistent for geminiviruses. Given that SlUPTG1 has a role in TLCV movement; down-regulation of *SlUPTG1* may provide a durable resistance by blocking the virus movement.