

Geminivirus Rep Protein Interferes with the Plant DNA Methylation Machinery and Suppresses Transcriptional Gene Silencing

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Cytosine methylation is an epigenetic mark that promotes gene silencing and plays an important role in genome defense against transposons and invading DNA viruses. So far just one geminiviral protein, C2 from *Tomato golden mosaic virus* (TGMV), *Beet curly top virus* (BCTV) and *Beet severe curly top virus* (BSCTV) and a betasatellite protein from *Tomato yellow leaf curl China virus* (TYLCCNV), have been shown to act as TGS suppressors by interfering with the proper functioning of the plant methylation cycle. We have found that geminiviral infection reduces the expression of the plant maintenance DNA methyltransferases, *MET1* and *CMT3*, in both, locally and systemically infected tissues of *Arabidopsis* and *Nicotianabenthamiana* and we demonstrated that the virus-mediated repression of these two maintenance DNA methyltransferases is widely spread among different geminivirus species (TGMV, *African cassava mosaic virus* (ACMV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV-Mld). Furthermore, we have identified Rep as the geminiviral protein responsible for the repression of *MET1* and *CMT3*, and another viral protein, C4, as an ancillary player in *MET1* downregulation. The presence of Rep, suppresses TGS of an *Arabidopsis* transgene and of host loci whose expression is strongly controlled by CG methylation. Additionally, bisulfite sequencing analyses showed that the expression of Rep caused a substantial reduction in the levels of DNA methylation at CG sites. Our findings suggest that Rep, the only viral protein essential for geminiviral replication, displays TGS suppressor activity through a mechanism distinct from the one thus far described for geminiviruses.