

GEMINIVIRUS REPLICATION PROTEIN IMPAIRS SUMO CONJUGATION OF PCNA AT TWO ACCEPTOR SITES

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Geminiviruses are plant viruses with circular, single-stranded DNA (ssDNA) genomes that infect a broad range of plants causing substantial crop diseases worldwide. They replicate in nuclei of infected cells by using host DNA replication machinery and an essential protein encoded in their genome designated Rep (replication-associated protein). This multifunctional protein induces the accumulation of the host factors involved in replication and it is capable of interacting with a lot of plant proteins including PCNA (Proliferating Cell Nuclear Antigen), a processivity factor that coordinates a wide range of processes involved in maintenance, duplication and transmission of the genome, and the sumoylation enzyme that conjugates SUMO to target proteins (SUMO-conjugating enzyme- SCE). PCNA modification by SUMO, and also ubiquitin, has long been known to be of key importance for determining how DNA damage is processed by the replisome and for maintenance of overall genome integrity. In yeast, PCNA sumoylation has been associated to DNA repair involving homologous recombination (HR). Previously, we reported that Rep ectopic expression does not result in broad changes in the sumoylation pattern of plant cells, but it modifies the sumoylation state of selected host proteins. In this work, we show, using a reconstituted sumoylation system in *Escherichia coli*, that tomato PCNA is sumoylated at two residues, K254 and K164, and that co-expression of the Rep protein suppresses PCNA sumoylation at these lysines. Finally, we confirm that PCNA is sumoylated and that Rep also interferes with PCNA sumoylation *in planta*.

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