GEMINIVIRUS REPLICATION PROTEIN DECREASES PCNA SUMOILATION AT TWO ACCEPTOR SITES

Blanca Sabarit[1], Manuel Arroyo-Mateos[1,2], Francesca Maio[2], Miguel Sánchez-Durán[1], Tábata Rosas-Díaz[1,4], Marcel Prins[3], Javier Ruiz -Albert[1], Ana P. Luna[1], Harrold A. van den Burg[2] and Eduardo R. Bejarano[1].

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", (IHSM-UMA-CSIC), Málaga, Spain. [2]Molecular Plant Pathology, Faculty of Science University of Amsterdam, The Netherlands. [3]Keygene NV, Wageningen, The Netherlands. [4] Shanghai Center for Plant Stress Biology/CAS Center for Excellence in Molecular Plant Science, Chinese Academy of Sciences, Shanghai, China.

INTRODUCTION

Geminiviruses are plant viruses with circular, single-stranded DNA (ssDNA) genomes that replicate in nuclei of mature plant cells by using plant replisome and the viral protein Rep. This viral protein interacts with diverse plant proteins, including PCNA (Proliferating Cell Nuclear Antigen) (Castillo *et al.*, 2003) and SCE1 (SUMO conjugating enzyme) (Castillo *et al*., 2004).

PCNA is a key factor for DNA replication, providing a platform that coordinates a wide range of processes involved in maintenance, duplication and transmission of the genome (fig. 1). Control of PCNA-interactions involves post-transcriptional modifications, such as ubiquitination and sumoilation. Sumoilation machinery includes a series of biochemical steps catalysed by a set of well conserved enzymes, comprising an activating enzyme E1, a conjugating enzyme E2 and a ligase E3 (fig. 2). SUMO can be covalently attached to lysine residues in a large number of proteins, regulating their target's interactions, localization and function. *Arabidopsis thaliana* has eight genes and one pseudogene. *AtSUMO1, 2, 3*, and *5* genes are expressed in *vivo*. Modification of PCNA by ubiquitin and SUMO are events that modulate the function of the protein (fig. 3).

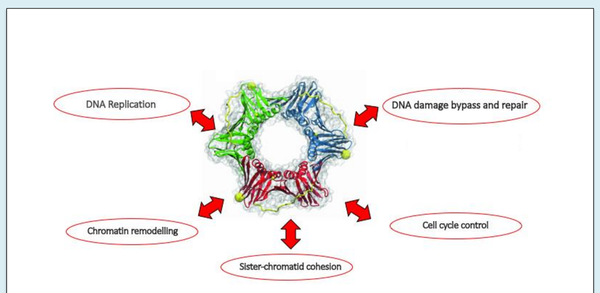


Figure 1. PCNA functions.

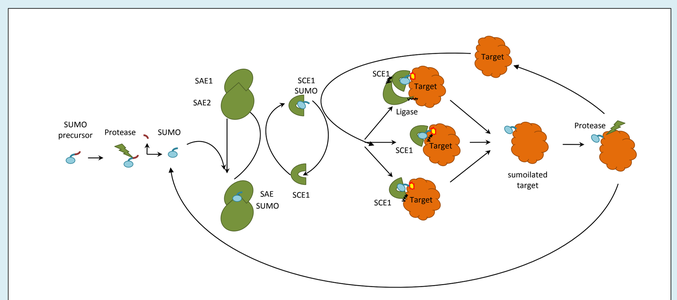


Figure 2. The sumoilation cycle.

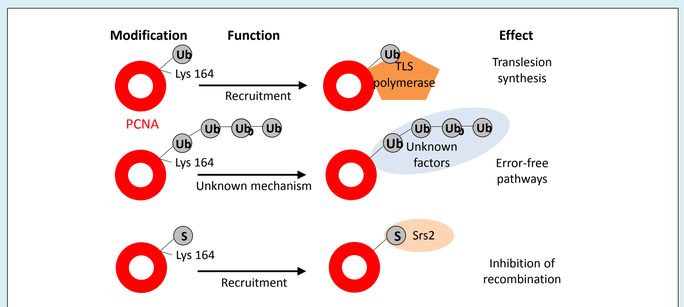
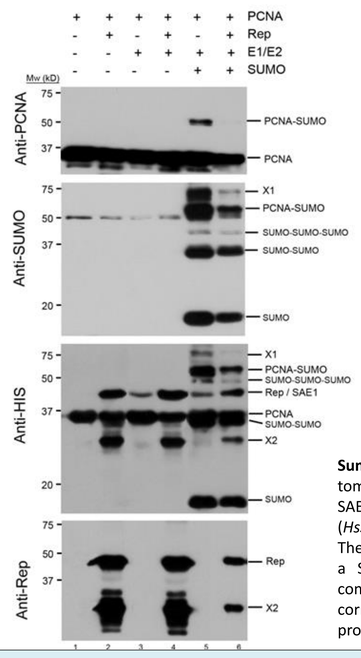


Figure 3. PCNA modifications during S phase and their effects on the genome (adapted from Strzalka *et al*., 2010).

Previously, we reported that Rep ectopic expression does not result in broad changes in the sumoilation pattern of plant cells, but it modifies the sumoilation state of selected host proteins. In this work, we show, using a reconstituted sumoilation system in *Escherichia coli,* that tomato PCNA is sumoilated at two residues, K254 and K164, and that co-expression of the Rep protein suppresses PCNA sumoilation at these lysines. Finally, we confirm that PCNA is sumoilated and that Rep also interferes with PCNA sumoilation *in planta.*

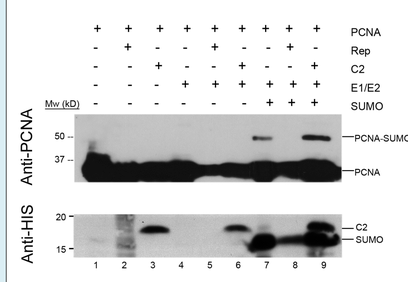
RESULTS

Rep expression reduces PCNA sumoilation

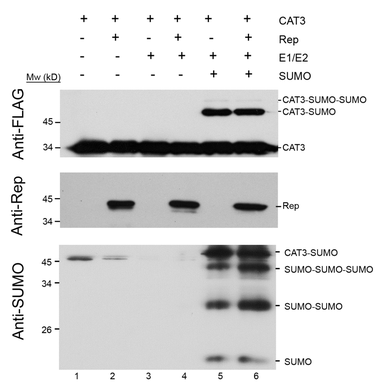


Sumoilation assay in *E.coli* co-expressing tomato PCNA, Rep, E1/E2 (mammalian SAE1, SAE2 and Ubc9), and SUMO (*HsSUMO1*).
The band labelled 'X1' could correspond to a SUMO tetramer or more likely to a complex of PCNA-2xSUMO. Band 'X2' corresponds to a truncated form of Rep protein.

Rep, but not C2, interferes only with sumoilation of PCNA but not of other proteins



Sumoilation assay in *E. coli* co-expressing tomato PCNA, Rep, **C2 from *Tomato yellow leaf curl Sardinia virus* (TYLCSV)**, E1/E2 (mammalian SAE1, SAE2 and Ubc9) and SUMO (*HsSUMO1*

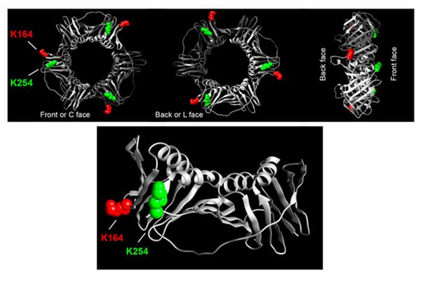


Sumoilation assay in *E. coli* co-expressing tomato PCNA, Rep, **Catalase3 (CAT3)**, E1/E2 (mammalian SAE1, SAE2 and Ubc9) and SUMO (*HsSUMO1*).

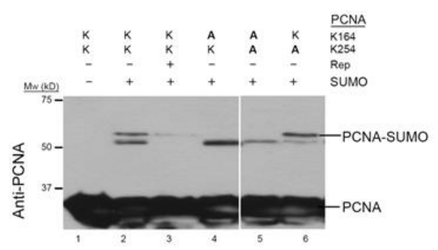
K164 and K245 residues are sumoilated in tomato PCNA

Tomato PCNA

```
MLELRLVQGSLLKKVLESIKDLVNDANFDCSATGFSLQAMDSSHVALVALLLRSEGFEHY     60
RCDRNISMGMNLTNMAKMLKCAGNDDIITIKADDGSDTVTFMFESPTQDKIADFEMKLMD    120
IDSEHLGIPEAEYHAIVRMPSAEFGRICKDLSSIGDTVVISVTKEGVKFSTRGDIGTANI    180
VCRQNTTVDKPEEATVIEMNEPVSLTFALRYLNSFTKASPLSNTVTISLSSELPVVVEYK    240
IAEMGYVRYYLAPKIEEDEEETKP                                         264
```

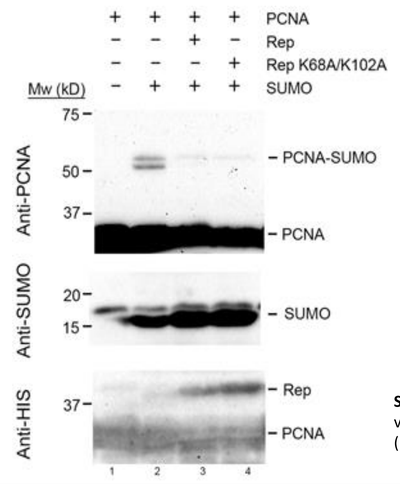


Sequence of tomato PCNA with residues K164 and K254 highlighted and the predicted 3D model of the structure of a tomato PCNA trimer (central panel) or a monomer (lower panel).



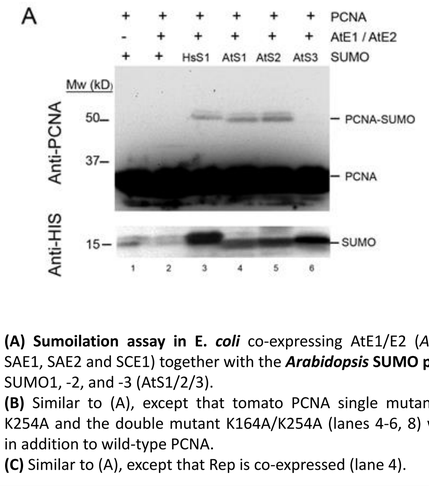
Sumoilation assay of tomato PCNA single mutants K164A, K254A and the double mutant K164A/K254A *in E. coli* while co-expressing Rep.

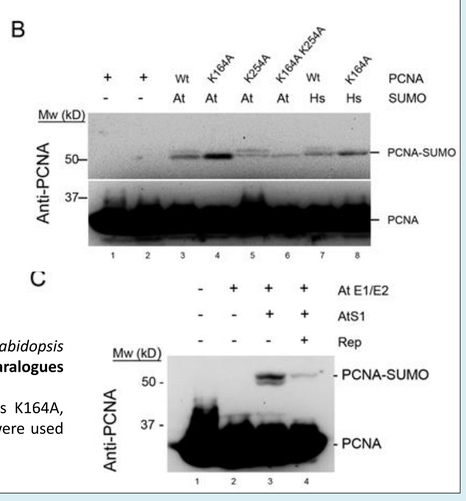
The Rep-SCE1 interaction is not essential to suppress PCNA sumoilation *in bacteria*



Sumoilation assay in *E.coli* expressing a Rep variant that fails to interact with SCE1 (K68A/K102A; lane 4).

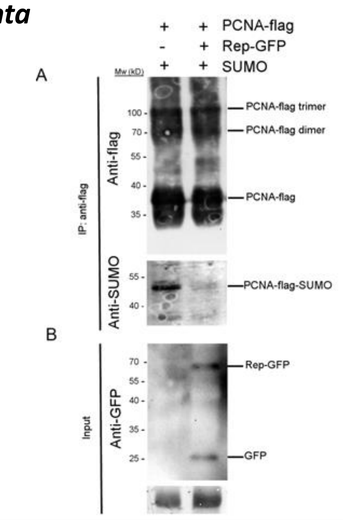
Arabidopsis SUMO conjugation enzymes modify the same lysine residues in tomato PCNA *in bacteria*





(A) Sumoilation assay in E. *coli* co-expressing AtE1/E2 (*Arabidopsis* SAE1, SAE2 and SCE1) together with the ***Arabidopsis* SUMO paralogues** SUMO1, -2, and -3 (AtS1/2/3).
(B) Similar to (A), except that tomato PCNA single mutants K164A, K254A and the double mutant K164A/K254A (lanes 4-6, 8) were used in addition to wild-type PCNA.
(C) Similar to (A), except that Rep is co-expressed (lane 4).

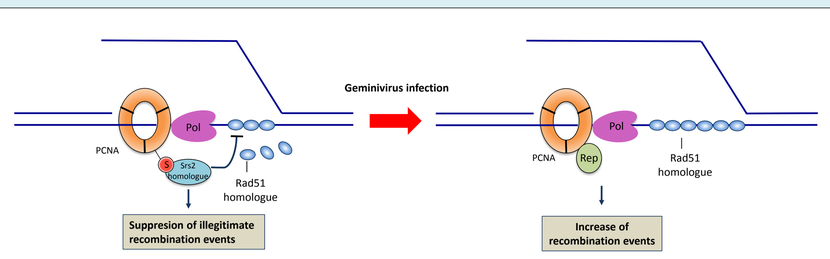
Rep from TYLCV compromises sumoilation of tomato PCNA *in planta*



(A) Flag-tagged tomato PCNA together with *Arabidopsis thaliana* SUMO1 was transiently expressed in *Nicotiana benthamiana* leaves in the presence or absence of GFP-tagged Rep from TYLCV. Total protein extracts were subjected to immunoprecipitation and proteins that co-eluted from the Flag affinity resin (IP:anti-Flag) were blotted with anti-Flag or anti-AtSUMO1 antibodies.
(B) Total protein extracts (input) were analyzed by immunoblotting using anti-GFP antibody to show accumulation of Rep-GFP and by Coomassie staining to confirm equal protein loading.

HYPOTHESIS

Geminivirus genomes undergo high levels of mutation, recombination and reassortment to increase viral diversity (Duffy & Holmes, 2008). In *Sacharomyces cerevisiae*, sumoilated PCNA binds Srs2, anti-recombinogenic protein that suppresses recombination during DNA replication by interfering with Rad51 and binding to DNA single strands formed in the replication fork (Veaute *et al*., 2003). Rep interferes with PCNA by decreasing PCNA-sumoilated levels, which could explain the increase of viral recombination rate.



Rep interference with PCNA increases recombination events. Model of our hypothesis based on *Sacharomyces cerevisiae* scenario. Modified from Mailand, 2013.

CONCLUSIONS

- Rep specifically interferes with PCNA sumoilation.
- PCNA lysines 164 and 254 are sumoilated and Rep interferes with the sumoilation of both residues.
- PCNA is sumoilated with AtSUMO1 and AtSUMO2 but not with AtSUMO3.

References:

- Castillo A. G. & Kong L. J. (2004). *J. Virol*.
- Castillo A. G., Collinet D., Kashoggi A., Bejarano E. R. (2003). *Virology*.
- Chymkowitch P., Nguea P. A., Aanes, H., Koehler, C., Thiede, B., Lorenz, S, et al. (2015) *Genome Res*.
- Duffy, S. & Holmes, E. C. (2008). *J Virol*.
- Kurepa J., Walker J. M., Smallet J., Gosink M. M., Davis S. J., Durham T. L., Sung D-Y and Vierstra D. (2003). *Journal of Biological Chemistry.*
- Mailand N., Gibbs-Seymour I. And Bekker-Jensen S. (2013) *Nat Rev Mol Cell Biol*.
- Strzalka W., and Ziemienowicz A. (2010). *Ann. Bot*.
- Strzalka W., Labecki P., Bartnicki F., Aggarwa, C., Rapala-Kozik M., Tani C., Tanaka K and Gabrys.(2012). *Journal of Experimental Botany*.
- Veaute X, Jeusset J, Soustelle C. *et al*. (2003). *Nature.*

Acknowledgement. This research was supported by a grant from the Spanish Ministerio de Ciencia y Tecnología (AGL2016-75819-C2-1-R). B. S. was awarded a Predoctoral Fellowship from the Spanish Ministerio de Economía y Competitividad.