Hijacking the plant DNA replication machinery - towards the structure of the viral replication initiator protein and its interaction partners

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Problems with begomoviruses have been heavily aggravated in recent years; in particular Tomato Yellow Leaf Curl Virus (TYLCV) causes severe damage on tomato and cucurbits impacting growers worldwide. One reason for the increase of this viral problem is climate change, as it has allowed the spread of the main vector for viral transmission, the whitefly *Bemisia tabaci,* which is now present in massive populations in more moderate climate zones including the Mediterranean. With the ban of pesticides and a lack of strong resistance genes in our crops, we urgently need alternative strategies to halt viral spread.

Since their viral genome lacks a gene encoding DNA polymerase activity, these viruses rely on host factors for their genome replication. The viral proteins thus need to (*i*) reprogram the host cell cycle to stimulate entry of the S phase, and to (*ii*) recruit the plant DNA-replication machinery to the viral DNA. Only one viral protein, the Replication initiator protein (Rep), is essential and sufficient to promote viral DNA replication inside plant cells. Rep interacts with a multitude of plant proteins including PCNA, a central processivity factor for DNA polymerases. Our goal is to elucidate the structure of Rep from TYLCV in complex with tomato PCNA. To obtain the 3D structure of Rep in complex with PCNA using X-ray crystallography, it will be critical to trap Rep in a fixed conformation and stoichiometry with PCNA. However, Rep displays conformational flexibility, and it can adopt different oligomeric states.

I will report on our efforts to purify recombinantly expressed Rep and PCNA proteins to obtain a complex suitable for crystallisation trials. Based on 3D-structure prediction tools, we are also in the process of designing Rep variants and truncations that likely trap the various protein domains of Rep in distinct conformations. By elucidating the Rep-PCNA interaction interface, we will gain invaluable insight in how Rep orchestrates DNA replication and identify contact residues at the interface of these two proteins. As this mechanism is possibly conserved across Circular Rep-encoding single-stranded DNA (CRESS-DNA) viruses, it will help us to understand rolling-circle DNA replication mediated by these viruses and to identify their weak spot.