Shedding light on the multiple functions of the geminivirus Replication initiator protein

Maio, F.

Publication date
2019

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Summary

Geminiviruses form a large and diverse family of ssDNA plant-infecting viruses that cause extensive crop losses worldwide. There are few resistance resources available for crop breeding while this virus family rapidly evolves due to recombination and error-prone replication. To attain durable and broad resistance against this devastating plant pathogen family, the most conserved geminiviral protein, Replication initiator protein (a.k.a Rep) is here studied. Rep is a multifunctional protein that is essential for viral replication. It orchestrates all layers of the viral DNA replication process by interacting with a plethora of host proteins. This thesis describes in great detail how Rep interferes and interacts with the plant sumoylation machinery, an essential post-translational modification (PTM). Sumoylation regulates the activity, localization and interaction network of hundreds of plant proteins by covalent attachment of Small ubiquitin-like modifier (SUMO) onto these target proteins. The data presented here provide new opportunities to engineer, e.g. genetically by induced mutagenesis or via gene editing, resistance against this virus family.

Geminiviruses depend on the host DNA replication machinery for their replication, as they do not encode their own DNA polymerase. Instead, Rep interacts with the host Proliferating cell nuclear antigen (PCNA) that forms a DNA clamp that acts as a processivity factor of eukaryotic DNA polymerases. In addition, Rep interacts with the E2 SUMO conjugation enzyme (SCE1). This interaction is essential for viral replication since mutations in Rep from *Tomato golden mosaic virus* (TGMV) that block SCE1-binding also impair viral DNA replication. Strikingly, ectopic overexpression of Rep modifies the sumoylation state of only a subset of host plant proteins. In **chapter 2**, it is established with a reconstituted sumoylation assay in bacteria that PCNA from tomato is sumoylated at two specific lysines *in vitro* and that Rep suppresses sumoylation at these lysines. We also detect, for the first time, sumoylation of PCNA *in planta* and we confirm that Rep also interferes with PCNA sumoylation *in planta*, which coincided with increased ubiquitylation of PCNA. Sumoylation of yeast PCNA at these lysines is known to inhibit DNA recombination activity, while ubiquitylation of one of these lysine residues (Lys164) results in recruitment of translesion DNA polymerases to DNA replication forks enabling DNA lesion bypass. These findings thus argue that Rep switches PCNA activity by reducing PCNA sumoylation, while promoting its ubiquitylation. Future work should reveal if this Rep-mediated switch of PCNA activity indeed promotes viral replication by inducing homologous recombination or recruiting e.g. DNA polymerases to viral DNA replication forks.

Mutations in two conserved lysine residues in the N-terminal half of Rep^{TGMV} were previously shown to disrupt the Rep-SCE1 interaction. **Chapter 3** describes that in the case of Rep from *Tomato yellow leaf curl virus* (TYLCV) these lysine residues are,

however, not essential for SCE1 binding. Instead, they control nuclear localization of Rep^{TYLCV} but not of Rep^{TGMV}. In support of this experimental data, we present a structural three-dimensional model of the N-terminal half of Rep, which reveals that mutating these lysines to alanines dramatically changes the surface charge of Rep^{TYLCV}, while the surface charge of Rep^{TGMV} remains largely intact. We also find that, independent of their role in nuclear localization, these lysine residues are essential for viral DNA replication activity of Rep^{TYLCV}, emphasizing again the extreme multi-functionality of this viral protein.

The results presented in chapter 3 signify that other residues in Rep^{TYLCV} must control its physical interaction with SCE1. **Chapter 4** reports on the identification of a conserved SUMO-interacting motif (SIM) in the C-terminal half of Rep. Mutations in this SIM not only disrupt its interaction with SUMO1 but also with SCE1. In support, we show that single residue mutations in SCE1 disrupt the interaction with SUMO and Rep. As some of these single residue substitutions yielded a biological active SCE1 protein in plants, these mutated variants of SCE1 might yield a source of resistance to geminiviruses. In these experiments, we also found that the Rep-SCE1 complex aggregates in specific nuclear compartments, named nuclear bodies (NBs). NBs formation depended on *(i)* SUMO conjugation activity, *(ii)* the SIM in Rep, and *(iii)* the second SUMO binding site of SCE1 – a non-covalent SUMO-binding pocket that is distal from the catalytic site of SCE1. Mutations in the SIM of Rep^{TYLCV} also inhibited Rep-mediated viral DNA replication, suggesting that the Rep-SUMO interaction is also essential for viral replication, albeit that it remains to be proven that ATPase activity is intact for these Rep variants.

The cell nucleus is a complex, highly structured and dynamic organelle that harbors a variety of discrete subnuclear compartments, collectively referred to as nuclear bodies (NBs). As the ternary complex formed by Rep-SCE1-SUMO aggregates in NBs, we investigated the nature and composition of these subnuclear structures. **Chapter 5** reports that the BiFC pairs formed by Rep-SCE1 and Rep-SUMO1 co-localize with regulators of the light-dependent growth and developmental pathway (photomorphogenesis) in so-called 'photobodies'. We found that Rep co-localizes with the master regulator of the dark response, COP1, a ubiquitin E3 ligase. This interaction appears not to be direct, but rather indirect via SCE1 and SUMO. Moreover, Rep localized in different sub-compartments of the nucleus depending on the light conditions provided, suggesting that its activity may be mediated by the light quality and intensity. In particular, blue light triggered a profound and rapid redistribution of Rep towards nuclear foci where the blue-light receptors Cryptochrome 1 and 2 (CRY1, CRY2) were also recruited in response to this blue light treatment. The Cryptochromes are known to inhibit COP1 activity by interacting with the COP1 complex in a light-dependent manner. By taking advantage of the

Arabidopsis double mutant *cry1;cry2*, it is then demonstrated that the geminivirus *Beet curly top virus* (BCTV) is capable of infecting the *cry1;cry2* double mutant with at a higher frequency than wild type plants (Col-0), demonstrating for the first time that blue light perception apparently has a role in viral replication.

Finally, **chapter 6** provides a fine example of the use of a high-throughput proteomics approach to identify new plant proteins that interact with Rep. In this work, Rep^{TYLCV} was tagged with GFP and this chimeric protein was overexpressed in tomato protoplasts, which allowed GFP-based affinity purifications of Rep-containing protein complexes. Co-purifying proteins were identified using tryptic digestion followed by tandem mass spectrometry. This yielded a list of novel candidate Rep interactors. Remarkably, two RNA-binding proteins from this list were confirmed to interact with Rep *in planta* using independent protein-protein interaction assays. This list contains new leads for developing geminivirus resistance, as interaction of these candidates with Rep might prove to be critical for viral replication and spread in the host plant. To conclude, the data presented in this PhD dissertation provide us with a deeper understanding of the Rep protein from different geminiviruses, while also yielding new leads and candidates for host processes that are manipulated by this extremely multifunctional viral protein.