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

Maio, F.

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Chapter 7

General Discussion

GENERAL DISCUSSION

The Replication initiator protein Rep is the most conserved geminiviral protein and it is the only one that is essential for the viral DNA replication process (Laufs et al. 1995; Nash et al. 2011). A deep understanding of the functions and interactions of this geminiviral protein is therefore of critical importance to be able to devise novel durable and broad resistance strategies against this family of devastating plant-infecting viruses based on the susceptibility gene approach. This PhD thesis uncovers novel details regarding *(i)* domains that control Rep localization and interactions, *(ii)* the interplay between Rep and the SUMO conjugation machinery, and *(iii)* novel host pathways and factors that are targeted by Rep. The discoveries here presented expose new perspectives on the interaction between the plant host and geminiviruses and thereby provide new leads to generate resistance in plants against these viruses.

The Rep protein contains several domains and this reflects its multifunctionality

The protein Rep performs most (if not all) of its multiple functions in the nucleus of the infected host cell. Surprisingly, the mechanism(s) by which the Rep protein is targeted to the plant nucleus has thus far been unknown. We discovered a region in the N-terminus of Rep from Tomato yellow leaf curl virus (TYLCV) that is required for its nuclear import (**Chapter 3**) (**Figure 1**). Substitution of the conserved lysine 67 to alanine, alone or combination with the same modification of other lysine residues, led to a profound shift of Rep^{TYLCV} accumulation outside of the plant nucleus. The region encompassing the lysines in position 67 and 101 constitutes a positively charged surface area that possibly acts as a nuclear localization signal, i.e. a peptide motif/patch that mediates the nuclear translocation of proteins by binding to nuclear cargo receptors, known as importins (karyopherins).

Importantly, the N-terminal half of Rep is characterized by the presence of several, often overlapping, protein domains involved in different functions (Ruhel and Chakraborty 2018). However, most of these functional domains have been described for Rep of the bipartite *Tomato golden mosaic virus* (TGMV) meaning that these domains remain to be validated in Rep proteins from other geminiviruses. In Rep^{TGMV} the corresponding lysine residues, K68 and K102, have been demonstrated to be required for two functions, namely *(i)* the interaction with the E2 SUMO conjugating enzyme SCE1 and *(ii)* replication of the viral DNA (Sánchez-Durán et al. 2011). Remarkably, we observed that, independent of their role in nuclear localization, for Rep^{TYLCV} these lysine residues are also essential for the viral DNA replication, but

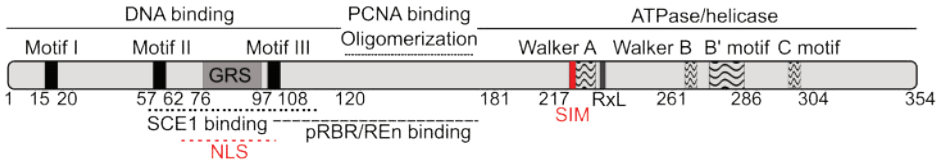

Figure 1. Diagram of Rep with its known and novel functional domains here identified
The motifs and protein domains of Rep^{TYLCV} identified in this thesis are depicted in red: the Nuclear localization signal (NLS, **Chapter 3**) and the SUMO-interacting motif (SIM, **Chapter 4**).

apparently not for SCE1 binding. On the other hand, Rep^{TGMV} variant in which K68 and K102 are mutated to alanine still retains its localization in the nucleus, suggesting that these residues are not required for nuclear import of Rep^{TGMV}. These results suggest that highly conserved residues or domains apparently may not have analogous roles in Rep proteins belonging to different geminiviruses and that the nuclear localization apparently depends on different residues in the various Rep proteins. Moreover, the question of which region of Rep^{TYLCV} is responsible for the interaction with SCE1 still remains unresolved. In **Chapter 4**, an answer to this query is found by the discovery of a new conserved peptide motif in the C-terminus of Rep that possesses the typical hallmarks of a SUMO-interacting motif (SIM) (**Figure 1**). This SIM indeed proves to be essential for the interaction of Rep^{TYLCV} with both SUMO1 and SCE1 *in planta.* An intact SIM was also required for Rep to activate amplification of viral DNA molecules, suggesting that SUMO-binding is essential for viral replication also in the case of Rep^{TYLCV}. Future studies will be required to address *(i)* whether the introduced mutations in the SIM affect the helicase and ATPase activity of Rep and *(ii)* whether the role of this SIM for the interaction with the SUMO conjugation machinery via SUMO is conserved in Reps from other geminiviruses.

To conclude, this thesis describes the discovery of two novel functional domains of Rep^{TYLCV} - a nuclear localization signal and a SIM - that unravel the mechanisms by which Rep is translocated to the host cell nucleus and engages with the SUMO conjugation machinery, respectively. In addition, our findings show a number of crucial points to keep in mind when studying this multifunctional viral protein: *(i)* one single residue or domain of Rep may contribute to more than one activity of this viral protein, *(ii)* common Rep features can be regulated by distinct domains in Rep proteins belonging to different geminiviruses, and *(iii)* conserved residues may display distinct functions in different Rep proteins.

Rep shows a range of distinct nuclear localizations that are potentially linked to its different functions

Geminivirus proteins, and in particular Rep, have evolved into multifunctional proteins to accommodate for the obligate (small) size of the viral DNA genome due to space restrictions in the virus particle (Fondong 2013). To date, little is known about the correlation between the distribution of Rep in the plant cell and its multiple activities. Our cell biology studies reveal in great detail that the localization of Rep into the plant cell nucleus is highly dynamic and depends on many factors such as individual Rep domains, interactions with other proteins and environmental cues (**Figure 2**).

First of all, we showed that Rep localizes in combination with SCE1 or SUMO1 in punctate nuclear structures, designated as nuclear bodies (NBs). These NBs displayed strong overlap with the position of the E3 ubiquitin ligase COP1, a master regulator of plant growth, and with other proteins involved in the photomorphogenesis pathway (*e.g.* SPA1 and phyB) (**Chapter 5**). The biological consequence of Rep recruitment in SUMO/COP1 NBs remains largely enigmatic, but it is potentially linked to Rep manipulating the sumoylation and/or ubiquitination status of certain host factors, e.g. PCNA and Histone 3 (Arroyo-Mateos et al. 2018; Kushwaha et al. 2017) (**Chapter 2**). This hypothesis is corroborated by the fact that Rep-SUMO NBs formation depends on *(i)* the physical interaction between the partners, *(ii)* on SUMO conjugation activity, and *(iii)* on non-covalent SUMO binding sites (SIMs) on both Rep and SCE1 (**Chapter 4**). Moreover, the SUMO conjugation machinery self-assembles in NBs that colocalize with COP1 photobodies (Mazur et al. 2019) and many components of these COP1 NBs are also SUMO conjugation targets, while they are also targeted for COP1-mediated proteasomal degradation. This suggests that Rep possibly interferes with the post-translational modification not only of DNA replication factors but also of regulators of the plant growth and developmental pathways, thus suggesting that geminiviruses might manipulate an additional, thus far unknown process via Rep.

Rep is also known to act as a transcription regulator and as suppressor of the RNA silencing (Eagle et al. 1994; Rodríguez-Negrete et al. 2013), but to date, none of the proven plant interactors of Rep has been associated with these activities. Here, two novel interaction partners of Rep are discovered, namely the RNA-binding EWS protein and a subunit of the THO complex, which are involved in transcriptional repression and nuclear export of RNA, respectively (**Chapter 6**). Remarkably, Rep resides in nuclear speckles when expressed as a part of a BiFC complex together with EWS. The nature and function of these NBs remains at large at this point. Based on predicted functions for EWS and the NBs phenotype (0-1-1.5 μm in size and ~20 NBs per nucleus), the Rep-EWS-dependent NBs possibly represent speckles where RNA-binding proteins accumulate and transcriptional regulation occurs (Sawyer and Dundr 2016; Shaw and Brown 2004).

In this thesis, data is presented that differences in the light spectrum can trigger a change in the distribution pattern of Rep in the nucleus and presumably in its function. In **Chapter 5**, it is presented that, in response to blue light, Rep is redistributed within minutes from the nucleoplasm to NBs and, at a later stage, to the nucleolar rim. In the case of Rep^{TYLCV}, these NBs concur with the subnuclear compartments that host the blue light photoreceptors Cryptochrome 1 and 2 (CRY1 and CRY2). Importantly, blue light exposure triggered a redistribution of different Rep proteins from a wide range of geminiviruses, albeit that it resulted in slightly different subnuclear patterns. Nevertheless, the majority of the tested Reps accumulate at the nucleolar rim after blue-light illumination. The consequences of Rep accumulation to different subnuclear compartments in response to blue light are yet undisclosed, but it is tempting to consider that these may be linked to a switch in Rep activity due to specific (changing) environmental conditions. From studies on human cell lines, it is evident that the nucleolar rim is rich in highly compacted heterochromatin and also harbours discrete NBs occupied by splicing factors, as well as RNA polymerase transcripts and RNA-binding proteins (Mao et al. 2011; Németh and Längst 2011). This perinucleolar heterochromatin ring is typically observed during the mid S phase of the cell cycle and primarily functions in replicating and maintaining repressive chromatin states (Zhang et al. 2007). Moreover, it is well known that nuclear architecture is under dynamic control by light (Bourbousse et al. 2015; Kaiserli et al. 2018). Light coordinates e.g. changes in

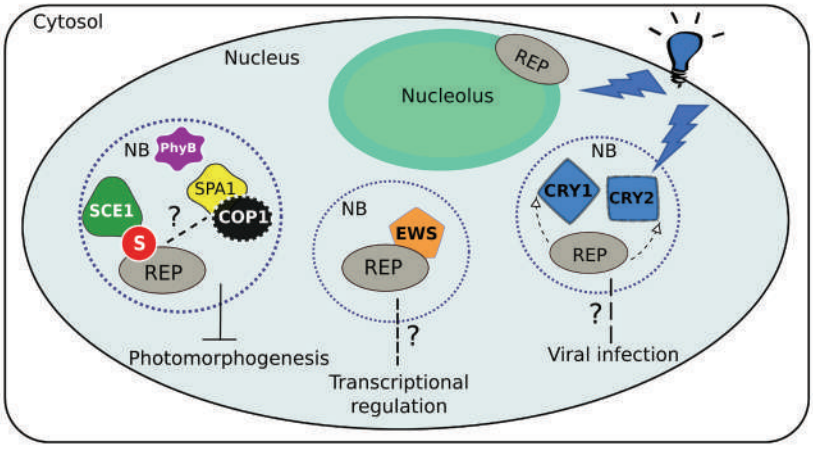

Figure 2. A model depicting the different localizations and interactions of Rep in the cell nucleus reported in this thesis

Rep interacts with SCE1 and SUMO1 in NBs where COP1 and regulators of photomorphogenesis are present. Rep also interacts with the RNA-binding protein EWS in different NBs, probably involved in transcriptional regulation. Upon blue light exposure, Rep relocalizes, at least in *N. benthamiana,* at the periphery of the nucleolus and in NBs that also contain the blue light-receptors CRY1 and CRY2.

chromatin remodelling, histone modifications and protein accumulation in nuclear photobodies, leading to transcriptional changes in the expression of genes involved in light signalling, metabolism and development (Jiao et al. 2007; Perrella and Kaiserli 2016; Tessadori et al. 2009). Interestingly, the photoreceptor CRY2 is a positive regulator of low light-induced chromatin decompaction (van Zanten et al. 2010). In relation to this data, it is also known that geminivirus infection induces the host cells to enter the S phase and alters nuclear architecture inducing plant chromatin condensation (Bass et al 2000). Moreover, Rep interacts with Histone 3 and manipulates the post-translational modification status of Histone 2B (Kushwaha et al. 2017). Thus, it can be hypothesized that the relocalization of Rep to the nucleolar rim and in Cryptochromes-containing NBs may be linked to the light-coordinated nuclear architecture remodelling and/or histone modification.

Rep engages with SUMO conjugation machinery to create an environment favorable for viral DNA replication

Over recent years, protein sumoylation has emerged as an important post-translational modification (PTM) that is manipulated in diverse ways by different viruses to suppress anti-viral responses and promote viral replication and viral pathogenesis (Lowrey et al. 2017; Mattoscio et al. 2013). Geminiviruses also engage with components of the plant sumoylation pathway, via the interaction between Rep and SCE1, but the precise biological role of this interplay has not been clarified. We here unveil for the first time that geminiviruses alter the sumoylation state of a specific host target, possibly to create an environment favorable for viral replication and propagation. **Chapter 2** illustrates that overexpression of Rep *in planta* lowers the sumoylation levels of Proliferating cell nuclear antigen (PCNA) - the processivity factor of eukaryotic DNA polymerase - while concomitantly enhancing the ubiquitination levels of PCNA. In yeast sumoylation of PCNA inhibits DNA homologous recombination, while ubiquitination results in recruitment of translesion DNA polymerases and activation of the error-free lesion bypass at a stalled replication fork (Lee and Myung 2008; Tsutakawa et al. 2015). Thus, Rep potentially mediates a switch of PCNA activity by affecting its post-translational modifications. The mechanism by which Rep suppresses PCNA sumoylation is yet unknown. A determining factor for the alteration of PCNA sumoylation potentially is the Rep non-covalent binding to SUMO via its SIM domain. A similar mechanism has previously been described for the BGLF4 protein of the human DNA virus Epstein-Barr (EBV). In the case of this virus, the SIM domains of BGLF4 are essential for BGLF4 to inhibit sumoylation of the EBV protein ZTA, likely via enhancement of phosphorylation of the target protein and recruitment of SUMO proteases (Li et

al. 2012). By binding to SUMO, the geminiviral Rep protein might interfere with the transfer of a SUMO moiety to SCE1 or to PCNA. The use of a Rep SIM mutant in a sumoylation assays will be required to confirm this hypothesis.

One of the consequences of SUMO attachment to a target is the promotion of inter and intra protein-protein interactions via SUMO-SIM interactions. To date, conjugation of a SUMO moiety to a geminiviral Rep protein has not been demonstrated, but we here show that Rep interacts non-covalently with SUMO via its predicted SIM domain in its C-terminal half (**Chapter 4**). This SIM possibly also mediates the interaction between Rep and several of its host protein partners. A SUMO-modified protein may gain additional affinities for a partner if this binding partner possesses SIMs on its surface that are accessible for SUMO-SIM interactions (Jentsch and Psakhye 2013; Kerscher 2007). Multiple SUMO-SIM interactions may then act synergistically and contribute to the formation of multiprotein complexes (Psakhye and Jentsch 2012). Given the fact that many Rep targets are SUMO substrates (**Chapter 1**) and that Rep is part of several multiprotein complexes (e.g. the viral replisome (Rizvi et al. 2015)), it would not be surprising that SIM-mediated interactions play a role in coordinating and stabilizing Rep binding to multiple components of the complexes. So far, Rep has been linked to interference of sumoylation of a specific target, i.e. PCNA. However, sumoylation is essential for the regulation of many cellular functions of the plant, including DNA repair and replication, cell division, expansion and differentiation (Augustine and Vierstra 2018; Hay 2005; Lamelí and Vázquez 2011). Possibly, geminiviruses promote SUMO modification of certain host factors via Rep and *de facto* Rep would thereby act as SUMO E3 ligase. This idea is corroborated by several observations. Firstly, SUMO E3 ligases commonly contain SIMs that play a role in increasing E3 ligase-dependent sumoylation of specific substrates (Reverter and Lima 2005; Yunus and Lima 2009). Secondly, several viruses encode a SUMO E3 ligase mimic that promotes (poly)sumoylation of host proteins (Muller and Dobner 2008; Pennella et al. 2010; Zhu et al. 2015). An interesting example is the K-bZIP protein encoded by the human Kaposi's sarcoma-associated herpesvirus. This protein is a SUMO E3 ligase and promotes sumoylation of Retinoblastoma protein in human cells in a SIM-dependent manner (Chang et al. 2010). Finally, Sánchez-Durán and co-workers showed that ectopic expression of geminiviral Rep induces accumulation of a certain SUMO protein conjugates in *N. benthamiana* leaves (Sánchez-Durán et al. 2011), suggesting that Rep may enhance sumoylation of specific individual host targets. A number of Rep-interactors are SUMO targets. For example, the human Retinoblastoma protein is sumoylated and its sumoylation is required for cell proliferation in human cells (Meng et al. 2016). The data here presented would thus warrant future studies to identify the proteins that become prone to sumoylation in the presence of Rep.

SCE1 is a potential target for novel resistance strategies against geminiviruses

An appealing opportunity to generate broad and durable resistance in crops against geminiviruses would be to be able to inhibit viral DNA replication, by impairing conserved and essential Rep-host protein interactions. To accomplish this, one would need to identify mutations in Rep-interacting proteins that prevent viral replication by suppressing interaction with Rep, while at the same time not disturbing essential host processes. In this thesis, SCE1 is studied as a putative target to generate geminivirus resistance. SCE1 is a highly conserved protein at the sequence level within the plant kingdom (Mazur et al. 2019; Novatchkova et al. 2012), with much of the surface exposed residues implicated in interactions with SUMO itself, the SUMO E1 activating enzyme, SUMO E3 ligase or substrates (Pichler et al. 2017). It interacts with geminiviral Rep and this interaction is necessary for viral replication and infection (Sánchez-Durán et al. 2011). However, at the beginning of this project, these data could only be addressed for the couple Rep^{TGMV}- *Nicotiana benthamiana* SCE1 and, moreover, the interaction surface on SCE1, to which Rep binds, remained unknown. We have here demonstrated that Rep-SCE1 interaction is conserved among Rep proteins belonging to a range of geminiviruses and it is necessary for viral replication also in the case of Rep^{TYLCV} (**Chapter 4**). In addition, we show that the second non-covalent SUMO binding site of SCE1 is required for the Rep-SCE1 interaction *in planta*. Single amino acid substitutions in the non-covalent SUMO binding site of SCE1 were sufficient to impair Rep binding to SCE1 and a number of these 'loss-of-interaction' SCE1 variants were shown to rescue embryo lethality of the Arabidopsis *sce1-5* knock-out mutant. Analyses revealed that some of the SCE1-complementation lines recovered the wild type-like phenotype in terms of rosette size, SCE1 expression (**Chapter 4**), inflorescence development and SUMO conjugates levels (data not shown), indicating that the mutated SCE1s retain their biological activity including SUMO E2 conjugation. Geminivirus infection assays on these Arabidopsis complementation lines are needed to verify whether introduction of single residue mutation in SCE1, next to disrupting the interaction between Rep and SCE1, also impairs viral DNA replication and thus confers plant resistance to geminiviruses. The identification of SCE1 variants that lose their interaction with viral Rep while still being biochemically active in planta is a first step towards a potentially durable and broad resistance against geminiviruses based on the putative viral susceptibility target SCE1. Additional studies are required to confirm that introduction of the 'loss-of-interaction' SCE1 variants in planta *(i)* does not compromise SUMO homeostasis and plant development, *(ii)* negatively impacts geminivirus replication, *(iii)* confers resistance against preferably a broad range of

geminiviruses in different (crop) plants.

Geminiviruses target the plant light-signaling pathway

Environmental cues, like temperature, circadian clock and light, influence plant processes such as growth and development, as well as plant-pathogen interactions (Gangappa and Kumar 2018; Roden and Ingle 2009; Yang et al. 2015). However, the role of environmental cues in virus replication has not been extensively explored for geminiviruses. In this thesis, the evidence that Rep engages with the plant photomorphogenic pathway and that environmental cues, specifically light conditions, alter geminiviral performances is presented for the first time.

In **Chapter 5**, it is shown that Rep co-localizes in the same subnuclear compartments that host COP1 and other regulators of the skoto/photomorphogenesis switch, i.e. the dark/light-mediated plant development programs that also integrate light quality (shade avoidance) and growth hormone signaling (gibberellic acid) (Hoecker 2017; Josse and Halliday 2008; Kim et al. 2017). The details about the interplay between Rep and the COP1 complex remain still unresolved, but it is possible that Rep, by modulating the activity of the master regulator of the plant growth and developmental pathway, might induce specific processes, such as endoreduplication, that favor viral replication. In darkness, the ubiquitin E3 ligase COP1 shuttles from the cytosol to the nucleus, where it targets positive transcriptional regulators of photomorphogenesis for degradation via the ubiquitin/26S proteasome pathway (Lau and Deng 2012; Stacey et al. 1999). As a result, seedlings exhibit etiolated growth characterized by an elongated hypocotyl, closed and undeveloped cotyledons and lack of pigmentation (Josse and Halliday 2008). This particular growth mode is linked to an increase in cell size due to an increase in the number of endoreduplication cycles (amplification of the genome in absence of mitosis) (Gendreau et al. 1998). Geminiviruses are known to increase endoreduplication in infected plant tissue and to replicate viral DNA during the S phase of the endocycle (Ascencio-Ibáñez et al. 2008). To date, the only known mechanism by which geminiviruses reprogram the host cell cycle is the Rep inhibition of the Retinoblastoma-related protein (RBR) (Kong et al. 2000). RBR interacts with E2F transcription factors to suppress expression of genes encoding host replication proteins (Desvoyes et al. 2006). Rep disrupts RBR-E2F complexes in order to allow transcription of E2F-target genes and as a result entry of the cell into the S phase. The data disclosed in **Chapter 5** provide clues that geminiviruses may potentially control host cell proliferation at yet another level by manipulating regulators of the dark/light-mediated plant growth pathway.

Understanding how geminivirus diseases develop in different environmental conditions and how geminiviruses integrate external stimuli into the viral replication

program is useful to manage geminivirus diseases. Thus far, only few studies investigated how light conditions could affect the life cycle of geminiviruses. We here expose that Cryptochrome-mediated blue light perception may have a role in geminiviral replication. In **Chapter 5**, it was shown that Arabidopsis mutant plants *cry1;cry2* are more readily infected by *Beet curly top virus* (BCTV). From previous studies, it is known that geminivirus *Abutilon Mosaic virus* (AbMV) infected plants show less mosaic symptoms under low light conditions (Jeske and Werz 1978) and cytological symptoms are dependent on circadian (Jeske 1986) and seasonal conditions (Schuchalter-Eicke and Jeske 1983). A low light regime also significantly reduces the severity of symptoms caused by two other begomoviruses in the host plant *Asystasia gangetica* (Wyant et al. 2015). However, whether light intensity and quality affect the virus life cycle directly or indirectly by modulating plant metabolism needs to be elucidated.

The light signaling cascade initiated by the Cryptochromes regulates different aspects of the plant cell cycle and plant growth and development such as de-etiolation (Ahmad and Cashmore 1993), flowering (Guo et al. 1998), guard cell development and stomatal opening (Kang et al. 2009; Mao et al. 2005), entrainment of the circadian clock (Somers et al. 1998), apical meristem activity (Lopez-Juez et al. 2008), shade avoidance (Keller et al. 2011) and programmed cell death (Danon et al. 2006). Given the diversity and number of processes regulated by the cryptochrome-mediated blue light perception, it is well conceivable that Rep targets these plant photoreceptors to induce an environment favorable for viral DNA replication. However, further studies are needed to investigate the mechanisms by which geminiviruses manipulate these sensors of external stimuli to their own advantage.

Global model of Rep manipulation of the host cell processes

As a summary, I have attempted to integrate the overall ideas that emerged from the data presented in thesis in a general model (**Figure 3**). To create a cellular environment that is favorable for viral DNA replication, geminiviruses induce infected cells, which are in a quiescent phase (G1 phase of the mitotic cycle or the G phase of the endocycle), to enter into the S phase in which, among other things, replication is promoted. The accepted mechanism by which geminiviruses regulate progression of the cell cycle into a replicative stage consists in the inhibition of the Retinoblastoma-related protein (RBR) via Rep interaction (Kong et al. 2000). Rep-RBR interaction relieves repression of the transcription factors E2F (E2Fa and E2Fc) and activates the expression of plant genes encoding host polymerases and accessory factors required

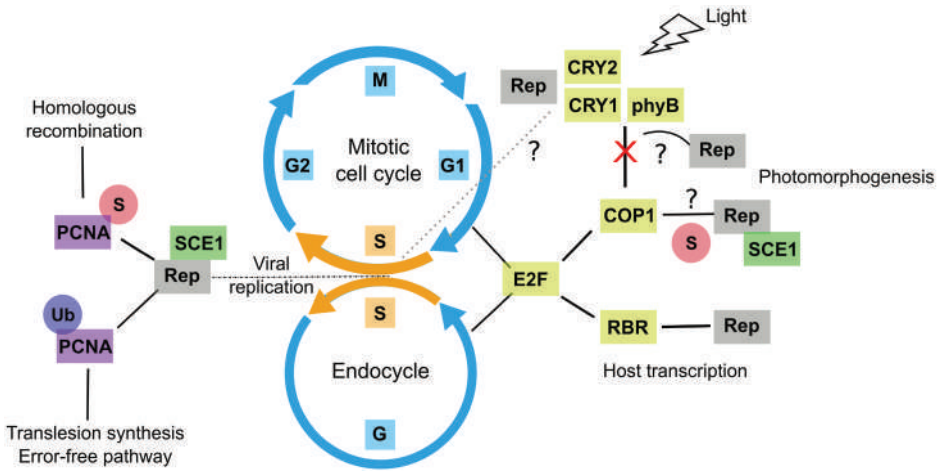

Figure 3. Global model of Rep manipulation of host pathways

Rep induces cells to enter the S phase of the mitotic cycle or of the endocycle by *(i)* regulation of the RBR/E2F pathway, and possibly *(ii)* manipulation of the COP1-dependent photomorphogenesis pathway, *(iii)* control of Cryptochrome-mediated processes. Rep also favor viral DNA replication by suppressing PCNA sumoylation while enhancing PCNA ubiquitination. Figure integrates the findings of this thesis in the models published by (Ascencio-Ibáñez et al. 2008; Hanley-Bowdoin et al. 2013)

for viral DNA replication (Desvoyes et al. 2006; Kong et al. 2000). Moreover, to prevent the mitotic cycle and promote the endocycle, geminiviruses suppress E2Fb activity (Ascencio-Ibáñez et al. 2008). E2F transcription factors are also regulated by environmental cues, such as light. Under dark conditions, E2Fb is marked by COP1 for degradation. Upon E2Fb degradation, E2Fc becomes the most abundant E2F and it binds and represses *DEL1* promoter. The decrease in transcription levels of the endocycle repressor *DEL1* leads to upregulation of endoreduplication (Berckmans et al. 2011). Based on those findings, it is possible that Rep promotes E2Fb COP1-mediated degradation via two putative mechanisms: *(i)* enhancement of COP1 activity via promotion of COP1 sumoylation; *(ii)* release of COP1 inhibition. The latter typically occurs via the activity of photoreceptors (phyB, CRY1 and CRY2) in response to light stimuli (**Chapter 5**). In addition, Rep possibly targets the Cryptochrome proteins directly in order to regulate other blue-light mediated growth and developmental pathways and as such ultimately promotes virus proliferation. Next to cell cycle regulation, Rep activates viral DNA replication via recruitment and manipulation of host replication factors, including PCNA (Bagewadi et al. 2004; Castillo et al. 2003). In **Chapter 2**, it is postulated that Rep impairs sumoylation of PCNA, which potentially suppresses interaction with the DNA helicase Srs2 and releases inhibition of homologous recombination, a mechanism that favors viral genome replication (Bisaro 1994; Pfander et al. 2005). At the same

time, Rep increases ubiquitination of PCNA (**Chapter 2**), which might result in recruitment of translesion DNA polymerases to DNA replication forks enabling DNA lesion bypass (Ciccia et al. 2012; Gali et al. 2012; Parker and Ulrich 2009).

Future perspectives

It is evident by now that the multitude of functions and interactions of the Rep protein makes Rep an indispensible 'Swiss army knife' that geminiviruses exploit to hijack the host cell processes and promote viral DNA replication. Several biochemical features of this geminivirus 'weapon' were known from previous studies, such as its DNA binding and nicking activity, helicase and ATPase activity as well as its ability to regulate gene expression and interact with host factors involved in DNA replication. In this thesis, novel details are revealed about the mechanisms by which Rep *(i)* is imported and localizes into the plant nucleus, *(ii)* interacts with and manipulates the plant SUMO conjugation machinery and *(iii)* potentially engages with the plant light-controlled growth pathway. These findings provide a starting point for further experiments aimed to fully characterize the interplay between Rep protein and the host processes.

For example, as Rep has been demonstrated to manipulate the post-translational modification status of specific host factors, we need to have a global picture of the changes in the PTM levels of the plant proteins upon geminivirus infection. Proteomic analyses of *e.g.* sumoylated and ubiquitinated host proteins in the context of geminivirus infection will help us to identify additional plant factors and pathways that are targeted by geminiviruses. It will also be necessary to better characterize the biological consequences and the exact molecular mechanism by which geminiviruses, via the Rep protein, modulate PTMs of host proteins. Such studies will not only provide crucial information about the outcomes of geminivirus-host interaction but also pinpoint potential novel resistance targets.

The here presented data about the possible involvement of blue-light perception in geminiviral DNA replication underscore the importance of better studying how environmental cues, such as light, affect the geminiviruses life cycle. Geminivirus infection assays *(i)* of plants impaired in the perception of specific light wavelength and *(ii)* in different light conditions should be therefore performed. Moreover, other environmental parameters, like temperature, circadian clock and plant hormones, need to be taken in account to understand how external stimuli are integrated during viral infection and influence geminivirus-host interaction. Such information may result in helpful measures that can be directly used to manage geminivirus diseases. Finally, it is essential to translate the mechanistic studies of geminivirus-host interaction in model organisms (Arabidopsis and *N. benthamiana* in our case) to

agricultural systems. For example, it needs to be validated whether SCE1 could be a suitable target for resistance against geminiviruses in important crops susceptible to geminiviruses such as tomato, pepper and other vegetables. An increasing amount of whole-genome sequence resources as well as new genetic and molecular technologies to manipulate non-model plants are now available and will facilitate the study and development of novel disease control strategies against these important plant pathogens.

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REFERENCES

Ahmad, M., and Cashmore, A. R. 1993. 'Hy4 Gene of a-Thaliana Encodes a Protein with Characteristics of a Blue-Light Photoreceptor', *Nature*, 366: 162-66.

Arroyo-Mateos, M., Sabarit, B., Maio, F., Sánchez-Durán, M. A., Rosas-Díaz, T., Prins, M., Ruiz-Albert, J., Luna, A. P., van den Burg, H. A., and Bejarano, E. R. 2018. 'Geminivirus Replication Protein Impairs SUMO Conjugation of PCNA at Two Acceptor Sites', *J Virol*, 92: e00611-18.

Ascencio-Ibáñez, J. T., Sozzani, R., Lee, T. J., Chu, T. M., Wolfinger, R. D., Cella, R., and Hanley-Bowdoin, L. 2008. 'Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection', *Plant Physiol*, 148: 436-54.

Augustine, R. C., and Vierstra, R. D. 2018. 'SUMOylation: re-wiring the plant nucleus during stress and development', *Curr Opin Plant Biol*, 45: 143-54.

Bagewadi, B., Chen, S., Lal, S. K., Choudhury, N. R., and Mukherjee, S. K. 2004. 'PCNA interacts with Indian mung bean yellow mosaic virus Rep and downregulates Rep activity', *J Virol*, 78: 11890-903.

Berckmans, B., Lammens, T., Van Den Daele, H., Magyar, Z., Bögre, L., and De Veylder, L. 2011. 'Light-dependent regulation of DEL1 is determined by the antagonistic action of E2Fb and E2Fc', *Plant Physiol*, 157: 1440-51.

Bisaro, D. M. 1994. 'Recombination in the Geminiviruses: Mechanisms for Maintaining Genome Size and Generating Genomic Diversity.' in J. Paszkowski (ed.), *Homologous Recombination and Gene Silencing in Plants* (Springer, Dordrecht: Dordrecht).

Bourbousse, C., Mestiri, I., Zabulon, G., Bourge, M., Formiggini, F., Koini, M. A., Brown, S. C., Fransz, P., Bowler, C., and Barneche, F. 2015. 'Light signaling controls nuclear architecture reorganization during seedling establishment', *Proc Natl Acad Sci U S A*, 112: E2836-44.

Castillo, A. G., Collinet, D., Deret, S., Kashoggi, A., and Bejarano, E. R. 2003. 'Dual interaction of plant PCNA with geminivirus replication accessory protein (Ren) and viral replication protein (Rep)', *Virology*, 312: 381-94.

Chang, P. C., Izumiya, Y., Wu, C. Y., Fitzgerald, L. D., Campbell, M., Ellison, T. J., Lam, K. S., Luciw, P. A., and Kung, H. J. 2010. 'Kaposi's Sarcoma-associated Herpesvirus (KSHV) Encodes a SUMO E3 ligase That Is SIM-dependent and SUMO-2/3-specific', *J Biol Chem*, 285: 5266-73.

Ciccia, A., Nimonkar, A. V., Hu, Y., Hajdu, I., Achar, Y. J., Izhar, L., Petit, S. A., Adamson, B., Yoon, J. C., Kowalczykowski, S. C., Livingston, D. M., Haracska, L., and Elledge, S. J. 2012. 'Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress', *Mol Cell*, 47: 396-409.

Danon, A., Coll, N. S., and Apel, K. 2006. 'Cryptochrome-1-dependent execution of programmed cell death induced by singlet oxygen in Arabidopsis thaliana', *Proc Natl Acad Sci U S A*, 103: 17036-41.

Desvoyes, B., Ramirez-Parra, E., Xie, Q., Chua, N. H., and Gutierrez, C. 2006. 'Cell type-specific role of the retinoblastoma/E2F pathway during Arabidopsis leaf development', *Plant Physiol*, 140: 67-80.

Eagle, P. A., Orozco, B. M., and Hanley-Bowdoin, L. 1994. 'A DNA sequence required for geminivirus replication also mediates transcriptional regulation', *Plant Cell*, 6: 1157-70.

Fondong, V. N. 2013. 'Geminivirus protein structure and function', *Mol Plant Pathol*, 14: 635-49.

Gali, H., Juhasz, S., Morocz, M., Hajdu, I., Fatyol, K., Szukacsov, V., Burkovics, P., and Haracska, L. 2012. 'Role of SUMO modification of human PCNA at stalled replication fork', *Nucleic Acids Res*, 40: 6049-59.

Gangappa, S. N., and Kumar, S. V. 2018. 'DET1 and COP1 Modulate the Coordination of Growth and Immunity in Response to Key Seasonal Signals in Arabidopsis', *Cell Rep*, 25: 29-37.

Gendreau, E., Höfte, H., Grandjean, O., Brown, S., and Traas, J. 1998. 'Phytochrome controls the number of endoreduplication cycles in the *Arabidopsis thaliana* hypocotyl', *Plant J*, 13: 221-30.

Guo, H. W., Yang, W. Y., Mockler, T. C., and Lin, C. T. 1998. 'Regulations of flowering time by Arabidopsis photoreceptors', *Science*, 279: 1360-63.

Hanley-Bowdoin, L., Bejarano, E. R., Robertson, D., and Mansoor, S. 2013. 'Geminiviruses: masters at redirecting and reprogramming plant processes', *Nat Rev Microbiol*, 11: 777-88.

Hay, R. T. 2005. 'SUMO: A history of modification', *Mol Cell*, 18: 1-12.

Hoecker, U. 2017. 'The activities of the E3 ubiquitin ligase COP1/SPA, a key repressor in light signaling', *Curr Opin Plant Biol*, 37: 63-69.

Jentsch, S., and Psakhye, I. 2013. 'Control of Nuclear Activities by Substrate-Selective and Protein-Group SUMOylation', *Annu Rev Genet*, 47: 167-86.

Jeske, H., and Werz, G. 1978. 'Influence of Light-Intensity on Pigment Composition and Ultrastructure of Plastids in Leaves of Diseased Abutilon-Sellowianum-Reg (Malvaceae)', *J Phytopathol*, 91: 1-13.

Jeske, H. 1986. 'The Detection of the Abutilon Mosaic-Virus Depends on the Experimental Daytime', *J Phytopathol*, 115: 243-56.

Jiao, Y. L., Lau, O. S., and Deng, X. W. 2007. 'Light-regulated transcriptional networks in higher plants', *Nat Rev Genet*, 8: 217-30.

Josse, E. M., and Halliday, K. J. 2008. 'Skotomorphogenesis: The Dark Side of Light Signalling', *Curr Biol*, 18: R1144-R46.

Kaiserli, E., Perrella, G., and Davidson, M. L. 2018. 'Light and temperature shape nuclear architecture and gene expression', *Curr Opin Plant Biol*, 45: 103-11.

Kang, C. Y., Lian, H. L., Wang, F. F., Huang, J. R., and Yang, H. Q. 2009. 'Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in Arabidopsis', *Plant Cell*, 21: 2624-41.

Keller, M. M., Jaillais, Y., Pedmale, U. V., Moreno, J. E., Chory, J., and Ballaré, C. L. 2011. 'Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades', *Plant J*, 67: 195-207.

Kerscher, O. 2007. 'SUMO junction-what's your function? New insights through SUMO-interacting motifs', *EMBO Rep*, 8: 550-5.

Kim, J. Y., Song, J. T., and Seo, H. S. 2017. 'COP1 regulates plant growth and development in response to light at the post-translational level', *J Exp Bot*, 68: 4737-48.

Kong, L. J., Orozco, B. M., Roe, J. L., Nagar, S., Ou, S., Feiler, H. S., Durfee, T., Miller, A. B., Gruissem, W., Robertson, D., and Hanley-Bowdoin, L. 2000. 'A geminivirus replication protein interacts with the retinoblastoma protein through a novel domain to determine symptoms and tissue specificity of infection in plants', *EMBO J*, 19: 3485-95.

Kushwaha, N. K., Mansi, and Chakraborty, S. 2017. 'The replication initiator protein of a geminivirus interacts with host monoubiquitination machinery and stimulates transcription of the viral genome', *PLoS Pathog*, 13: e1006587.

Lamelí, H., and Vázquez, M. 2011. 'Emerging roles of the SUMO pathway in development', *Cell Mol Life Sci*, 68: 4045-64.

Lau, O. S., and Deng, X. W. 2012. 'The photomorphogenic repressors COP1 and DET1: 20 years later', *Trends Plant Sci*, 17: 584-93.

Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S. G., Schell, J., and Gronenborn, B. 1995. 'In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus', *Proc Natl Acad Sci U S A*, 92: 3879-83.

Lee, K. Y., and Myung, K. J. 2008. 'PCNA modifications for regulation of post-replication repair pathways', *Mol Cells*, 26: 5-11.

Li, R. W., Wang, L. Y., Liao, G. L., Guzzo, C. M., Matunis, M. J., Zhu, H., and Hayward, S. D. 2012. 'SUMO Binding by the Epstein-Barr Virus Protein Kinase BGLF4 Is Crucial for BGLF4 Function', *J Virol*, 86: 5412-21.

Lopez-Juez, E., Dillon, E., Magyar, Z., Khan, S., Hazeldine, S., de Jager, S. M., Murray, J. A. H., Beemster, G. T. S., Bogre, L., and Shanahan, H. 2008. 'Distinct light-initiated gene expression and cell cycle programs in the shoot apex and cotyledons of Arabidopsis', *Plant Cell*, 20: 947-68.

Lowrey, A. J., Cramblet, W., and Bentz, G. L. 2017. 'Viral manipulation of the cellular sumoylation machinery', *Cell Commun Signal*, 15: 27.

Mao, J., Zhang, Y. C., Sang, Y., Li, Q. H., and Yang, H. Q. 2005. 'A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening', *Proc Natl Acad Sci U S A*, 102: 12270-75.

Mao, Y. S., Zhang, B., and Spector, D. L. 2011. 'Biogenesis and function of nuclear bodies', *Trends Genet*, 27: 295-306.

Mattoscio, D., Segré, C. V., and Chiocca, S. 2013. 'Viral manipulation of cellular protein conjugation pathways: The SUMO lesson', *World J Virol*, 2: 79-90.

Mazur, M. J., Kwaaitaal, M., Mateos, M. A., Maio, F., Kini, R. K., Prins, M., and van den Burg, H. A. 2019. 'The SUMO Conjugation Complex Self-Assembles into Nuclear Bodies Independent of SIZ1 and COP1', *Plant Physiol*, 179: 168-83.

Meng, F. X., Qian, J., Yue, H., Li, X. F., and Xue, K. 2016. 'SUMOylation of Rb enhances its binding with CDK2 and phosphorylation at early G1 phase', *Cell Cycle*, 15: 1724-32.

Muller, S., and Dobner, T. 2008. 'The adenovirus E1B-55K oncoprotein induces SUMO modification of p53', *Cell Cycle*, 7: 754-58.

Nash, T. E., Dallas, M. B., Reyes, M. I., Buhrman, G. K., Ascencio-Ibañez, J. T., and Hanley-Bowdoin, L. 2011. 'Functional analysis of a novel motif conserved across geminivirus Rep proteins', *J Virol*, 85: 1182-92.

Németh, A., and Längst, G. 2011. 'Genome organization in and around the nucleolus', *Trends Genet*, 27: 149-56.

Novatchkova, M., Tomanov, K., Hofmann, K., Stuible, H. P., and Bachmair, A. 2012. 'Update on sumoylation: defining core components of the plant SUMO conjugation system by phylogenetic comparison', *New Phytol*, 195: 23-31.

Parker, J. L., and Ulrich, H. D. 2009. 'Mechanistic analysis of PCNA poly-ubiquitylation by the ubiquitin

protein ligases Rad18 and Rad5', *EMBO J*, 28: 3657-66.

Pennella, M. A., Liu, Y., Woo, J. L., Kim, C. A., and Berk, A. J. 2010. 'Adenovirus E1B 55-Kilodalton Protein Is a p53-SUMO1 E3 Ligase That Represses p53 and Stimulates Its Nuclear Export through Interactions with Promyelocytic Leukemia Nuclear Bodies', *J Virol*, 84: 12210-25.

Perrella, G., and Kaiserli, E. 2016. 'Light behind the curtain: photoregulation of nuclear architecture and chromatin dynamics in plants', *New Phytol*, 212: 908-19.

Pfander, B., Moldovan, G. L., Sacher, M., Hoege, C., and Jentsch, S. 2005. 'SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase', *Nature*, 436: 428-33.

Pichler, A., Fatouros, C., Lee, H., and Eisenhardt, N. 2017. 'SUMO conjugation - a mechanistic view', *Biomol Concepts*, 8: 13-36.

Psakhye, I., and Jentsch, S. 2012. 'Protein Group Modification and Synergy in the SUMO Pathway as Exemplified in DNA Repair', *Cell*, 151: 807-20.

Reverter, D., and Lima, C. D. 2005. 'Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex', *Nature*, 435: 687-92.

Rizvi, I., Choudhury, N. R., and Tuteja, N. 2015. 'Insights into the functional characteristics of geminivirus rolling-circle replication initiator protein and its interaction with host factors affecting viral DNA replication', *Arch Virol*, 160: 375-87.

Roden, L. C., and Ingle, R. A. 2009. 'Lights, Rhythms, Infection: The Role of Light and the Circadian Clock in Determining the Outcome of Plant-Pathogen Interactions', *Plant Cell*, 21: 2546-52.

Rodríguez-Negrete, E., Lozano-Durán, R., Piedra-Aguilera, A., Cruzado, L., Bejarano, E. R., and Castillo, A. G. 2013. 'Geminivirus Rep protein interferes with the plant DNA methylation machinery and suppresses transcriptional gene silencing', *New Phytol*, 199: 464-75.

Ruhel, Rajrani, and Chakraborty, Supriya. 2018. 'Multifunctional roles of geminivirus encoded replication initiator protein', *VirusDis*: 1-8.

Sánchez-Durán, M. A., Dallas, M. B., Ascencio-Ibañez, J. T., Reyes, M. I., Arroyo-Mateos, M., Ruiz-Albert, J., Hanley-Bowdoin, L., and Bejarano, E. R. 2011. 'Interaction between geminivirus replication protein and the SUMO-conjugating enzyme is required for viral infection', *J Virol*, 85: 9789-800.

Sawyer, I. A., and Dundr, M. 2016. 'Nuclear bodies: Built to boost', *J Cell Biol*, 213: 509-11.

Schuchalter-Eicke, G., and Jeske, H. 1983. 'Seasonal Changes in the Chloroplast Ultrastructure in Abutilon Mosaic-Virus (Abmv) Infected Abutilon Spec (Malvaceae)', *J Phytophatol*, 108: 172-84.

Shaw, P. J., and Brown, J. W. 2004. 'Plant nuclear bodies', *Curr Opin Plant Biol*, 7: 614-20.

Somers, D. E., Devlin, P. F., and Kay, S. A. 1998. 'Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock', *Science*, 282: 1488-90.

Stacey, M. G., Hicks, S. N., and von Arnim, A. G. 1999. 'Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of Arabidopsis COP1', *Plant Cell*, 11: 349-63.

Tessadori, F., van Zanten, M., Pavlova, P., Clifton, R., Pontvianne, F., Snoek, L. B., Millenaar, F. F., Schulkes, R. K., van Driel, R., Voesenek, L. A., Spillane, C., Pikaard, C. S., Fransz, P., and Peeters, A. J. 2009. 'Phytochrome B and histone deacetylase 6 control light-induced chromatin compaction in Arabidopsis thaliana', *PLoS Genet*, 5: e1000638.

Tsutakawa, S. E., Yan, C., Xu, X., Weinacht, C. P., Freudenthal, B. D., Yang, K., Zhuang, Z., Washington, M. T., Tainer, J. A., and Ivanov, I. 2015. 'Structurally distinct ubiquitin- and sumo-modified PCNA: implications for their distinct roles in the DNA damage response', *Structure*, 23: 724-33.

van Zanten, M., Tessadori, F., McLoughlin, F., Smith, R., Millenaar, F. F., van Driel, R., Voesenek, L. A., Peeters, A. J., and Fransz, P. 2010. 'Photoreceptors CRYTOCHROME2 and phytochrome B control chromatin compaction in Arabidopsis', *Plant Physiol*, 154: 1686-96.

Wyant, P., Strohmeier, S., Fischer, A., Schäfer, B., Briddon, R. W., Krenz, B., and Jeske, H. 2015. 'Light-

dependent segregation of begomoviruses in Asystasia gangetica leaves', *Virus Res*, 195: 225-35.

Yang, Y. X., Wang, M. M., Yin, Y. L., Onac, E., Zhou, G. F., Peng, S., Xia, X. J., Shi, K., Yu, J. Q., and Zhou, Y. H. 2015. 'RNA-seq analysis reveals the role of red light in resistance against Pseudomonas syringae pv. tomato DC3000 in tomato plants', *BMC Genomics*, 16: 120.

Yunus, A. A., and Lima, C. D. 2009. 'Structure of the Siz/PIAS SUMO E3 Ligase Siz1 and Determinants Required for SUMO Modification of PCNA', *Mol Cell*, 35: 669-82.

Zhang, L. F., Huynh, K. D., and Lee, J. T. 2007. 'Perinucleolar targeting of the inactive X during S phase: Evidence for a role in the maintenance of silencing', *Cell*, 129: 693-706.

Zhu, Z. X., Yang, F., Zhang, K. S., Cao, W. J., Jin, Y., Wang, G. Q., Mao, R. Q., Li, D., Guo, J. H., Liu, X. T., and Zheng, H. X. 2015. 'Comparative Proteomic Analysis of Wild-Type and SAP Domain Mutant Foot-and-Mouth Disease Virus-Infected Porcine Cells Identifies the Ubiquitin-Activating Enzyme UBE1 Required for Virus Replication', *J Proteome Res*, 14: 4194-206.