Ubiquitin and plant viruses, let's play together!

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Abstract
Over the last few decades, posttranslational modification of proteins by the addition of ubiquitin (Ub) and degradation by the Ubiquitin proteasome system (UPS) has emerged as a critical regulatory process in virtually all aspects of cell biology, including host/pathogen interactions. Recent findings point to the importance of Ub-related pathways during viral infection in plants. Some viruses have been shown to hijack the UPS system, most likely for their own benefit. In other cases, viral proteins are themselves the targets of the UPS. The exquisite versatility of Ub conjugation, combined with the amazing abilities of viruses to manipulate regulatory pathways, hold promises for exciting discoveries in the future.
1- Introduction

Over the last thirty years, posttranslational modification of proteins by ubiquitin (Ub) and degradation by the Ubiquitin proteasome system (UPS) has emerged as a major regulatory process in virtually all aspects of cell biology (Glickman and Ciechanover, 2002). Ub-mediated degradation is widely conserved across the eukaryotic kingdoms and, judging from the large number of Arabidopsis thaliana genes involved in Ub-dependent protein turnover, as well as accumulating biochemical or genetic studies, protein degradation by the UPS plays a central role in many processes in plants (Bachmair et al., 2001, Vierstra, 2009).

The involvement of the UPS in the signaling and regulation of interactions between plants and pathogens is also becoming increasingly clear (reviewed in Zeng et al., 2006; Dreher and Callis, 2007; Citovsky et al., 2009, Dielen et al., 2010, Marino et al., 2012). Given the importance of Ub attachment in regulating the fate and function of proteins, it is not surprising that a wide range of pathogens, particularly viruses, have found many ways to exploit and interfere with the UPS (Shackelford and Pagano, 2005, Randow and Lehner, 2009, Isaacson and Ploegh, 2009).

In the case of plant viruses, a connection between the Ub system and virus infection was suggested by early observations indicating that perturbation of the Ub conjugation pathway altered plant responses to Tobacco mosaic virus (TMV) infection (Becker et al., 1993). However, appreciation of the full involvement of the UPS in the regulation of plant-virus interactions has long been limited by the extreme paucity of known targets. It is only recently that a number of viral proteins acting in these processes have been identified, and possible mechanisms proposed. It is likely that many more examples remain to be discovered.

In some cases, viral proteins appear to usurp the UPS by targeting cellular proteins for degradation, presumably to the benefit of the virus. In other instances, viral proteins are themselves the target of Ub-conjugation events. At present, it is not clear whether these ubiquitination events are obligatory steps in the viral life cycle or whether they represent failed attempts of the host cell to interfere with viral multiplication. Our recent data indicate that some of these conjugation events can be reversed by dedicated viral proteins, highlighting their remarkable plasticity, and suggesting that their reversal may constitute an additional level of regulation during viral infection.

Because such findings point to the importance of ubiquitin-related pathways during viral infection in plants, the aim of this review is to summarize current knowledge and discuss different aspects of the involvement of the UPS in plant-virus interactions. The exquisite versatility of Ub conjugation, combined with the remarkable capacity of viruses to manipulate regulatory pathways, hold promises for exciting discoveries in the future.
2- The Ub proteasome Pathway

Ub is a 76-residue protein that is highly conserved throughout the eukaryotic kingdom. Attachment of Ub to cellular proteins (referred to as ubiquitination or ubiquitylation) is involved in the regulation of many signaling pathways, and also plays an important role in protein homeostasis (Glickman and Ciechanover, 2002). Three distinct phases in the ubiquitination process are controlled by three classes of enzymes (Figure 1): i) activation of Ub via a ubiquitin activating enzyme (E1)—during which Ub is transferred onto the E1; ii) transfer of Ub from the E1 enzyme to a ubiquitin conjugating enzyme (E2); iii) transfer of Ub from the E2 enzyme onto the protein substrate—a process achieved by an E3 ligase, which coordinates ubiquitination by providing a binding platform for E2 enzymes and specific substrates. E3 ligases constitute a large family of proteins that mediate the specificity of substrate ubiquitination, and as such they constitute very appealing targets, not only for pharmaceutical companies, but also for viral pathogens.

Ub is linked to the target protein via an isopeptide bond between its C-terminal Gly residue and an acceptor amino acid of the target protein substrate—in most cases a lysine residue. This modification (mono-ubiquitination) can then be extended by ligation of additional Ub molecules. In that case, a lysine residue of Ub serves as a conjugation site for the addition of the next Ub molecule, generating poly-ubiquitinated chains. Any of the seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) present in Ub can serve as an acceptor, resulting in different branching patterns with different topologies (Xu and Peng, 2006). Depending on the type of ubiquitination event (mono- versus poly-ubiquitination), the length of the chain (< or > 4 Ub molecules), and the type of chain-branching, ubiquitination of proteins may have different functions in the cell. Whereas the most studied polyubiquitin K48 linkage is associated with degradation by the proteasome, alternative ubiquitination events are destined for other cellular processes, such as subcellular localization, protein activation or protein-protein interactions, thus illustrating the exquisite versatility of Ub conjugation (Ikeda and Dikic, 2008). Specific functions associated with non-canonical chain types remain so far undetermined in plants.

Ubiquitination can be reversed by the action of enzymes known as ubiquitin hydrolases or deubiquitinating enzymes (DUBs) (Komander et al., 2009). Most of these enzymes are cysteine proteinases that cleave isopeptidase bonds. They either trim poly-Ub chains or remove them from substrate proteins, thus contributing to the reversal of signaling events, to protein stabilization, and to Ub homeostasis in cells. The importance of DUBs in the regulation of cellular processes is only now beginning to emerge.
3- Hijacking of the UPS system by plant viruses

Members of several different groups of plant viruses have been shown to exploit the UPS system, in all likelihood for their own benefit, using a variety of mechanisms (Table 1). Reports have described their ability to induce, inhibit, or modify the specificity of Ub-related host enzymes—in particular E3 ligases. In addition, an example of a virally-encoded DUB was reported recently, indicating that plant viruses may also—like their animal counterparts—encode for Ub-related enzymes. Such mechanisms may be beneficial to the virus either by creating a more favorable cellular environment, or by inhibiting host defense mechanisms. Similar strategies have also been reported in the case of bacterial or fungal pathogens, as reviewed in this issue.

3-1 Induction of expression of host UPS-related proteins

A number of proteins related to the UPS—including Ub itself—have been reported to be induced upon viral infection (Aranda et al., 1996, Whitham et al., 2003, Takizawa et al., 2005, Ye et al., 2011) but in most cases it is unclear whether increased expression is necessary to degrade specific cellular or viral proteins, or whether it is part of the general cellular stress response due to viral protein expression and accumulation (Aparicio et al., 2005, Vitale and Boston, 2008, Sugio et al., 2009).

One example, however, points to the importance of the induction of a specific E3 ligase for the control of viral infection. In the geminivirus Beet severe curly top virus (BSCTV), expression of the C4 protein—a major determinant of pathogenesis affecting cell division—was found to promote expression of the RING-type E3 ligase RKP (related to KPC1) (Lai et al., 2009). Interestingly, RKP regulates the cell cycle through degradation of the cell cycle inhibitors ICK/KRPs (Ren et al., 2008), and modification of the accumulation level of these protein substrates was also observed in BSCTV-infected plants. As impairment of viral multiplication was observed in plants knocked-down for RKP as well as in those overexpressing ICK/KRP, Lai et al. (2009) thus proposed a convincing model in which C4 affects BSCTV infection by regulating the host cell cycle—to which viral replication is coupled—through controlling accumulation of ICK/KRPs.

3-2 Inhibition of host E2 enzymes

In the Geminivirus Cotton leaf curl Multan virus (CLCuMV), the βC1 protein—a pathogenicity factor encoded by the satellite β DNA - was reported to interact with the host Ubiquitin-conjugating (E2) enzyme S1UBC3 (Eini et al., 2009). Overexpression of βC1 in transgenic plants led to a decrease in the global accumulation level of polyubiquitinated proteins, supporting the idea that βC1 inhibits the Ub-conjugation step in a non-specific manner. The interaction of βC1 with S1UBC3 was also found to correlate with the severity of symptoms in plants—which are reminiscent of those observed upon perturbation of the Ub
system in plants (Bachmair et al., 1990)—and thus may reflect the numerous perturbations in developmental and hormonal signaling pathways that are regulated by the UPS (Kelley and Estelle, 2012).

3.3 Usurping host E3 ligases through recruitment of F-box containing proteins

There is increasing evidence that usurping of host E3 ligases is a common strategy used by plant viruses. In all cases reported so far, viruses were observed to hijack a particularly versatile class of E3 Ub ligases, designated SCF complexes (Lechner et al., 2006) (Figure 2). Such complexes are comprised of four components: RBX1, Cullin and SKP1, which form a conserved scaffold that assembles with one particular F-box protein. This class of E3 is the most prevalent in plants, with more than 700 F-box proteins having been identified through genome analysis and domain homology (Gagne et al., 2002). F-box proteins serve as substrate-specific adapters, binding to SKP1 through their F-box motif and recruiting protein targets to the core ubiquitination complex by means of a specific protein-protein interaction domain.

Encoding a protein bearing an F-box motif may allow viruses to hijack the host E3 ligase core complex to promote degradation of specific key cellular proteins. The identification of such proteins remains challenging, as their substrates are expected to be ubiquitinated and degraded as a result of their association with the enzyme.

The first example of this putative viral hijacking was reported for the multicomponent single-stranded DNA nanovirus Faba bean necrotic yellows virus (FBNYV). The virally encoded CLINK (cell cycle LINK) protein contains an F-box motif that interacts with SKP1 in vitro and in vivo, suggesting its involvement in a bona-fide SCF complex (Aronson et al., 2000). Interestingly, CLINK also harbors a motif known to interact with the retinoblastoma tumor suppressor protein pRB (Aronson et al., 2000). Through alteration of pRB activity, the virus acquires the capacity to modify cell cycling and to force cells into DNA synthesis (Lageix et al., 2007), thus creating a cellular environment favorable for efficient replication of the viral genome. However, whether CLINK inactivates pRB by targeting its degradation, as described for animal papillomavirus, remains to be established.

Another example of viral proteins bearing an F-box motif are the P0 proteins encoded by the Poleroviruses Beet western yellows virus (BWYV) and Cucurbit aphid-borne yellows virus (CABYV) (Pazhouhandeh et al. 2006), which are potent suppressors of RNA silencing—one of the major antiviral defense system in plants (reviewed in Ding, 2010). Both proteins were found to interact with the Arabidopsis homologues of SKP1, ASK1 and ASK2. Interestingly, the suppressor activity of P0 was found to correlate with the functionality of its F-box, supporting the idea that P0 targets an essential component of the host RNA silencing pathway for degradation (Pazhouhandeh et al. 2006). This component was identified as being
ARGONAUTE1 (AGO1) (Baumberger et al., 2007; Bortolamiol et al., 2007)—the core component of the RISC complex involved in the RNA silencing pathway (reviewed in Vaucheret, 2008). Interaction of P0 with AGO1 is proposed to interfere with its assembly within RISC complexes (Csorba et al., 2010), leading to its degradation by an as yet unclear, proteasome-independent mechanism (Baumberger et al., 2007).

Such findings are particularly exciting because they not only exemplify a novel viral strategy to counteract antiviral plant defenses, but also raise the provocative possibility of an interplay between protein degradation and the RNA silencing pathway.

The capacity of viral silencing suppressors to affect AGO1 protein stability has been reported for two additional examples: the enamovirus Pea enation mosaic virus-1 (PEMV-1) protein P0 (Fusaro et al., 2012), and the potexvirus Potato virus X (PVX) protein P25 (Chiu et al., 2010). In the case of PVX, targeting of AGO1 appeared dependent on proteasome activity (Chiu et al., 2010).

Knock-down of expression of the SKP1 subunit in the SCF complex was found to severely impair BWYV infection (Pazhouhandeh et al. 2006), and to cause a delay in PVX systemic infection (Ye at al., 2011), thus confirming the importance of this type of E3 ligase in viral infectivity. However, further studies are required to determine whether the effect on PVX infectivity relates to AGO1-mediated suppression of RNA silencing or to another—possibly coordinated—effect of the UPS on viral cell-to-cell movement (see below, Ye and Verchot, 2011).

More recently, an alternative pathway was proposed, where the virus itself does not encode an F-box protein, but instead interacts with a plant F-box protein. The protein P25 encoded by the Benyvirus Beet necrotic yellow vein virus (BNYVV) has been described as a pathogenicity factor leading to the appearance of necrosis in susceptible sugar beets. Yeast two-hybrid experiments evidenced the interaction of P25 with a number of candidate proteins involved in ubiquitination (Thiel and Varrelmann, 2009), in particular a protein containing an F-box domain and two kelch repeats. The biological relevance of BNYVV P25 in terms of degradation remains unclear, but rather than P25 being a substrate of this host F-box protein, it has been proposed that P25 might inhibit the interaction between the SKP1 homologue ASK1 and the F-box protein, thereby altering proper target recognition, and leading to cell necrosis in an as yet undefined manner (Thiel et al., 2012).

**3-4 Preventing degradation by impairment of host E3 ligases**

In contrast to the examples given above, which exemplify a viral strategy aimed at destroying cellular proteins, an alternative strategy consists of protecting from degradation host proteins that are usually unstable.

In this context, S-adenosyl-methionine decarboxylase 1 (SAMDC1) was identified as an interaction partner of the geminivirus BSCTV silencing suppressor protein C2 (Zhang et
al., 2011). SAMDC1 proteasomal degradation appears inhibited by BSCTV C2—a process which in turn impacts host and viral DNA methylation, providing a way to negatively regulate the gene-silencing mediated antiviral defense mechanism in planta and to facilitate viral multiplication (Zhang et al., 2011).

How BSCTV C2 achieves stabilization of SAMDC1 is presently unknown. However, data obtained in the case of other geminiviruses—Tomato yellow leaf curl Sardinia virus (TYLCSV), Tomato yellow leaf curl virus (TYLCV) and Beet curly top virus (BCTV) — support the idea that the C2/L2 protein can usurp the UPS by targeting a broad range of E3 ligases all at once, by acting on their neddylation/rubylation status (Lozano-Duran et al., 2011a). Nedd8 (also called RUB1 in plants) is a Ub-like protein (UbL), whose reversible conjugation to the Cullin subunit of E3 ligase is required for its activation (Hotton and Callis, 2008) (Figure 2). RUB1 is conjugated to cullins via specific enzymes in a manner similar to that of Ub conjugation, while deconjugation is achieved by the de-RUBylation activity of the COP9 signalosome complex (CSN) (Schwechheimer and Isono, 2010). Expression of C2/L2 proteins in transgenic Arabidopsis plants was found to compromise the activity of the CSN over CUL1, which thus accumulates in its RUBylated form. As a consequence, plant pathways that are regulated by these SCF complexes are altered (Lozano-Duran et al., 2011a). Given the pleiotropy of physiological and developmental processes regulated by SCFs (Hua and Vierstra, 2011), this capability of Geminivirus proteins to interfere with the activity of SCF complexes is an extremely powerful strategy to interfere with plant physiology, and in particular with hormone-mediated plant defense responses. Processes related to jasmonate biosynthesis and perception were found to be the major targets of TYLCSV C2 protein (Lozano-Duran et al., 2011a).

Even more attractive is the observation that particular SCF complexes can escape this inhibition, provided that the corresponding specific F-box protein is overexpressed—a process that indeed appears to occur during geminivirus infection (Lozano-Duran and Bejarano, 2011). These results thus raise the tantalizing idea that geminiviruses may have the ability to regulate distinct SCF complexes both positively and negatively simultaneously by playing with their constituent subunits. It should be noted that the use of global approaches has been instrumental in producing these exciting findings.

3-5 Preventing degradation by virally encoded deubiquitinating enzymes

Ubiquitination processes can be reversed by the action of deubiquitinating enzymes (DUBs). Although several DUBs have now been described in animal viruses, it is only recently that such an activity was demonstrated to be encoded by the plant virus Turnip yellow mosaic virus (TYMV) (Chenon et al., 2012). TYMV DUB activity is carried by the cysteine proteinase domain of the replication protein, which is also involved in endoproteolytic processing of viral proteins. It displays structural homologies with the ovarian
tumor (OTU) protein but, in contrast to the homologous DUBs encoded by animal viruses, TYMV DUB does not exhibit a global effect on the accumulation level of polyubiquitinated proteins. Instead, it was reported to specifically target the viral polymerase—shown previously to be a proteasomal substrate (Camborde et al., 2010) (see below)—leading to its de-ubiquitination and subsequent stabilization. It is worth noting that many plant viruses encode cysteine proteinase domains, and a subset of *Flexiviridae* members also contains a proteinase domain with homologies to cellular OTU-like proteins (Makarova et al., 2000, Martelli et al., 2007). If these viral enzymes could act as DUBs, this would suggest an important function for such an activity also in these viruses.

3-6 Sumoylation of viral replication proteins

SUMO (small ubiquitin-related modifier) is a UbL protein that can be covalently attached to Lys residues of target proteins via an enzymatic cascade mechanistically similar to that of Ub (Ulrich, 2009). The functions of SUMO-conjugation (referred to as SUMOylation) vary depending on the target protein, as a major consequence of SUMOylation is to inhibit, modify or enable protein / protein interactions. Because SUMO is conjugated to Lys residues, it can also compete with Ub-conjugation, thereby modulating protein stability and subcellular localization (Ulrich, 2009).

The importance of SUMOylation is illustrated by the cases of Geminivirus *Tomato golden mosaic virus* (TGMV), *African cassava mosaic virus* (ACMV) and TYLSCV, whose replication proteins *Rep* interact with the host SUMO-conjugating enzyme E1 (Castillo et al., 2004). Altering SUMO expression (either positively or negatively) strongly reduced viral replication (Castillo et al., 2004). Further analysis revealed that the interaction between *Rep* and SUMO E1 is required for viral DNA replication and viral infectivity. Overexpression of *Rep* did not alter the general SUMOylation pattern of plant proteins, but as few additional conjugated proteins were detected, it was proposed that modulation of SUMOylation may be limited to specific host proteins, which remain to be defined (Sanchez-Duran et al., 2011).

4- Viral protein targets : is Ub friend or foe ?

Information from genome-wide genetic or proteomic screens (Kushner et al., 2003, Panavas et al., 2005, Serviene et al., 2006, Li et al., 2008, Gancarz et al., 2011, Lozano-Duran et al., 2011b) support the idea that ubiquitination of viral proteins is important for viral multiplication. Direct demonstration of Ub conjugation of viral proteins has also been reported in a few cases (described further below: Table 2). Yet our current understanding of the precise role of this post-translational modification is limited: in addition to modification of protein turn-over by proteasomal degradation, Ub conjugation may affect protein localization, may serve as a molecular switch between different functions, or affect the ability of the viral proteins to interact with specific host factors. This diversity of functions stems from the
myriad ways in which target proteins can be modified, e.g., mono-, multi-mono- or poly-
ubiquitination (Figure 1) and, in the latter case, also in the type of Ub chain linkages or their
length (Ikeda and Dikic, 2008). However, such information is scarce in the case of plant or
plant viral proteins, and thus the role(s) of such post-translational modification(s) is often
undetermined. The interpretation of such data is complicated further by the fact that UPS
degradation can correspond either to the regulation of functional proteins carrying specific
destruction signals, or to direct the removal of damaged, misfolded, or overexpressed
proteins. Such processes are reversible—thanks to viral or cellular DUBs—and can also be
competed out by UbL proteins (such as SUMO), which may form conjugates with specific
target lysine residues, thus leading to protection from Ub-mediated proteolysis. Finally,
whether viruses take any advantage of these degradation processes, or whether they
correspond solely to cellular defense responses, or both, is still unclear.

4-1 Mono-ubiquitination of viral replication proteins

Viral replication is the central step of the infection cycle, with the rapid production of
huge numbers of viral progeny in the infected cell. Viral genome replication requires the
assembly of replication complexes featuring the close association of both viral and host
components in particular subcellular compartments.

In the case of Tombusvirus *Tomato bushy stunt virus* (TBSV), Cdc34p E2 Ub-
conjugating enzyme was described as a novel component of the viral replication complex, and
as critical for efficient replicase activity (Li et al., 2008). Cdc34p was found to interact
directly with TBSV p33 replication protein, leading to its mono- and bi-ubiquitination (Li et
al., 2008). Ubiquitination of p33 seems to have no effect on its metabolic stability (Barajas et
al., 2010). Instead, it was found to contribute to the interaction with ESCRT (endosomal
sorting complexes required for transport) proteins (Barajas et al., 2010), whose temporary
recruitment to sites of viral replication is required for optimal replicase activity and protection
of the viral RNA template (Barajas et al., 2009a). Ubiquitination of p33 thus appears to be
required for the proper targeting of TBSV replication complexes.

4-2 Poly-ubiquitination and degradation of viral replication proteins

The assembly of viral replication complexes depends on many critical interactions
between various partners. Modifying the proper stoichiometry of replication complex subunits
via selective degradation is thus likely to affect the outcome of viral replication. In some
instances proteasome subunits, E3 ligases, or cellular DUBs have been shown to affect the
efficiency of viral replication, but such effects could not be linked directly to degradation of
viral proteins by the UPS, and could possibly be due to indirect effects caused by cellular
stress and/or perturbation of Ub homeostasis (Barajas et al., 2009b, Yamaji et al., 2010,
Gancarz et al., 2011, Wang et al., 2011).
More direct evidence for the involvement of the UPS in the metabolic stability and accumulation level of viral replication proteins, and hence in the efficiency of viral replication, has been demonstrated recently in the case of TYMV.

TYMV 66K polymerase accumulates in infected cells (Prod’homme et al. 2001), but this accumulation is transient due to degradation of 66K by the UPS at late time points in viral infection (Camborde et al., 2010). Degradation of the polymerase appeared to be a limiting factor during viral infection, but as the polymerase bears a degradation signal—identified as a PEST-like sequence—that is conserved among tymoviruses (Héricourt et al., 2000, Camborde et al., 2010), it is likely that ubiquitination and/or proteasomal degradation of the polymerase is important for regulation of viral replication. Several hypotheses have been put forward (Camborde et al., 2010): i) low levels of polymerase may help maintain the integrity of the viral genome as polymerase crowding favors viral RNA recombination; ii) the amount of polymerase available may constitute a switch either in the replication process itself, or iii) during other steps of the viral multiplication cycle; or iv) ubiquitination as a means of escape from the host surveillance mechanisms.

However, the most surprising finding was the recent report that such ubiquitination and degradation processes can be counteracted by another TYMV-encoded replication protein bearing a DUB activity (Chenon et al., 2012). Because proteasomal degradation of 66K could have been avoided easily by mutation of the PEST sequence during evolution, this result points to the importance of the dynamics and reversibility of ubiquitination events, and underscores the idea that the virus has evolved to take advantage of both ubiquitination and de-ubiquitination events for implementing precise temporal and/or spatial control of its life cycle.

4-3 Polyubiquitination and degradation of viral movement proteins

Following infection of an individual cell, plant viruses spread from cell to cell through intercellular connections, the plasmodesmata, by exploiting virus-encoded movement proteins (MPs) (Lucas, 2006, Ueki and Citovsky, 2011). MPs are thought to form complexes with the viral genome, docking it to plasmodesmata, where they then increase plasmodesmatal permeability to allow its transport into neighbouring cells. Strikingly, a number of viral MPs accumulate only transiently during the early to mid-stages of viral infection, and the timing of expression appears critical for viral infection (Maule, 1991). The selective degradation of proteins is a recurrent theme in regulatory mechanisms involving timing control (Glickman and Ciechanover, 2002), and the UPS degradation of virus MPs indeed appears to be a rather common phenomenon.

TMV MP was the first viral MP reported to be degraded in vivo, as proteasome inhibitors lead to increased stability of this protein, which then accumulates in a polyubiquitinated form, presumably in the ER (Reichel and Beachy, 2000). TYMV MP was also shown to be very unstable in vitro, to be polyubiquitinated and to be a substrate of the
proteasome (Drugeon and Jupin, 2002). More recently, *Potato leafroll virus* (PLRV) MP, and the PVX protein TGBp3 were also shown to be degraded by the proteasome (Vogel et al., 2007, Ju et al., 2008). In the latter case, translocating TGBp3 from the ER to the cytoplasm for degradation, was demonstrated to involve an ER-associated protein degradation (ERAD) pathway, a component of the protein quality control system that normally eliminates misfolded or unassembled proteins from the ER (reviewed in Smith et al., 2011). This was so far unprecedented in plant viruses (Ju et al., 2008, Ye et al., 2011).

A number of hypotheses have been put forward to explain the relevance of such observations during viral infection. UPS degradation may be considered as a specific host cell defense pathway against viral infection as MP is central to the spread of viruses—and in some instances also appears to be a suppressor of RNA silencing (Voinnet et al., 2000, Chen et al., 2004), thus constituting a good target for plant defense responses. Such a hypothesis would be consistent with the observation that improved TMV trafficking functions correlate with evasion of the host degradation pathway (Gillespie et al., 2002).

In the case of PVX, however, degradation does not seem to be a limiting factor in viral infection (Ju et al., 2008), and it has been proposed that ERAD induction is primarily a stress response, preventing the cytotoxicity and cell death that is linked to ER stress (Ye et al., 2011). In turn, this process may also be considered as a viral strategy to maintain host viability, which is both in the interest of the virus and its host cell. That induction of the unfolded protein response (UPR) as a consequence of continued ER stress may also be used by PVX as a means to stimulate AGO1 degradation, as reported above, constitutes an interesting possibility, although not yet demonstrated (Ye and Verchot, 2011).

### 4.4 Mono-ubiquitination of viral structural proteins

Mono-ubiquitination of the TMV coat protein was described almost 25 years ago (Dunigan et al., 1988), after observing that a minor fraction (estimated at an average frequency of one per virion) was conjugated to a host protein. This observation was soon extended to the structural proteins of other viruses (i.e. *Barley stripe mosaic virus* (BSMV), *Brome mosaic virus* (BMV), *Cowpea mosaic virus* (CPMV), *Cowpea severe mosaic virus* (CPSMV), and *Satellite panicum mosaic virus* (SPMV) (Hazelwood and Zaitlin, 1990), but the significance of these observations has not been elucidated to date.

### 4.5 Polyubiquitination and degradation of viral structural proteins

During viral infection, structural proteins are produced in enormous amounts, and can constitute up to 20 % of total cell protein content. As the error-rate of viral polymerases generates mutations at high frequency, it is not unexpected that misfolded and insoluble viral structural proteins are produced during viral multiplication. It was shown for TMV that such proteins are massively polyubiquitinated (Jockusch and Wiegand, 2003). As functional
proteins are not targeted, these ubiquitination events are likely to correspond to a cellular protein response to stress (Sugio et al., 2009), rather than a specific regulatory process.

In contrast, the precursor of the Caulimovirus *Cauliflower mosaic virus* (CaMV) coat protein (CP) was reported to contain three specific instability determinants (Karsies et al., 2001), which make this protein likely to be the target of a regulatory process. One uncharacterized degradation signal targets the CP precursor for degradation by the proteasome, whereas the other two, which display characteristics of PEST-related sequences (Rechsteiner and Rogers, 1996) seem to induce a proteasomal-independent degradation pathway. Although mutation of the degradation signals affected viral infectivity, how these data relate to our current knowledge of the CaMV multiplication cycle remains unclear.

5- Concluding remarks

Ubiquitin can intervene at each and every step of the viral multiplication cycle, and in turn viruses have developed many tools to usurp the UPS. There is converging evidence on the importance of the UPS in the regulation of viral infection, and it is likely that the development of molecular or protein tools (i.e. tagged-versions of Ub, high-affinity Ub-binding traps or sensitive antibodies), will precipitate many more reports. Efforts to identify specific degradation signals may help discriminate between specific degradation and clearing of misfolded proteins. As mentioned above, ubiquitinated substrate proteins are not necessarily targeted for degradation, as mono-ubiquitination or "atypical" chain types have various other functions. Characterization of the linkage type of Ub chains and, determining their functions in plants, as well as the existence of possible cross-talk between Ub and UbL(s), or between Ub and other post-translational modifications, are missing at the moment and there is an urgent need to explore these questions in the near future.

Regarding usurping of the UPS, this appears to be done mostly by hijacking E3 ligases; the identification and characterization of host substrates will confirm the importance of this tactic, either for promoting a favorable cellular environment or for blocking the activation of defense mechanisms. In this respect, it will not be surprising if key plant defense regulators are identified as substrates of the UPS upon viral hijacking. This is likely to provide exciting new insights into the molecular mechanisms associated with plant-virus interactions.

In recent years, our appreciation of the dynamic aspects of Ub modifications has increased dramatically. It now appears that viruses probably benefit from the spatial and temporal fine-tuning occasioned by such reversible and versatile modification. Understanding where and when ubiquitination of host and viral targets operates, could thus turn out to be as important as identifying the substrates themselves.

Finally, the main question that remains open is what is the function of such modifications in the viral life cycle: do they represent a viral strategy to enhance infectivity, a
host defense reaction, and a combination of the two? To decipher this entanglement, a system-wide analysis of the ubiquitination networks that are linked to viral infection will be necessary. This will require a combination of modern proteomics approaches, reverse genetic screens, and biochemical characterization of enzyme activities. We expect such studies to provide mechanistic insights into the complexity of virus infection, but also to reveal how these mechanisms could be exploited to target viruses. The development of small molecules that interfere with the activity of the Ub-related enzymes that are essential for viral infectivity or are misregulated in disease may thus possibly be exploited for the development of new antiviral strategies.

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Figure Legends

Figure 1: The Ubiquitin Proteasome system—UPS.
Ubiquitination of a target protein substrate occurs through the sequential action of three classes of enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). It can be reversed by the action of deubiquitinating enzymes (DUBs). By reiterative rounds of ubiquitination, polymeric Ub chains can be generated, whose function depends on the type of Ub-chains attached to the target substrate.

Figure 2: Schematic representation of an SCF-type E3 ligase.
The generic architecture of an SCF complex is shown. The F-box protein serves as a substrate recognition subunit. Activity of the SCF complexes is regulated by conjugation of the cullin subunit to the UbL RUB1. The COP9 signalosome contributes to this regulation due to its de-RUBylating activity.
### Plant virus proteins hijacking the UPS

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**Table 1: Usurping of the UPS by plant virus proteins**
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Table 2: Plant virus proteins targeted by the UPS