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REVIEW

The ubiquitin-proteasome system in positive-strand RNA virus infection

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SUMMARY

Positive-stranded RNA viruses, like many other viruses, have evolved to exploit the host cellular machinery to their own advantage. In eukaryotic cells, the ubiquitin-proteasome system (UPS) that serves as the major intracellular pathway for protein degradation and modification plays a crucial role in the regulation of many fundamental cellular functions. A growing amount of evidence has suggested that the UPS can be utilized by positive-sense RNA viruses. The UPS eliminates excess viral proteins that prevent viral replication and modulates the function of viral proteins through post-translational modification mediated by ubiquitin or ubiquitin-like proteins. This review will discuss the current understanding of how positive RNA viruses have evolved various mechanisms to usurp the host UPS to modulate the function and stability of viral proteins. In addition to the pro-viral function, UPS-mediated viral protein degradation may also constitute a host defense process against some positive-stranded RNA viral infections. This issue will also be discussed in the current review. Copyright © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

Positive-strand RNA viruses cover more than one-third of all virus genera and infect a wide range of hosts, for example, the plants, animals, and humans. Examples of this class of viruses include poliovirus, coxsackievirus, EMCV, HCV, HAV, SARS-CoV, West Nile virus, and dengue fever virus [1]. Positive-strand RNA viruses, either enveloped or non-enveloped, consist of a single positive-strand of RNA acting as mRNA to direct the synthesis of viral proteins. Viral structural proteins make up the viral capsid, and non-structural proteins, such as viral proteases and RNA-dependent RNA polymerases, function to process the viral polyprotein and catalyze the synthesis of the viral progeny genomes, respectively.

Similar to other viral pathogens, positive-strand RNA viruses utilize and subvert the host cellular machinery to support their life cycle. Among the many host pathways that can be modulated by positive strand RNA viruses, the ubiquitin-proteasome system (UPS), the major intracellular protein degradation pathway, has recently received considerable attention because the interaction between viruses and the UPS has been found to play important roles in many aspects of the viral life cycle [2,3]. For positive-strand RNA viruses, it has been shown that an appropriate stoichiometric ratio of viral structural to non-structural proteins is essential for effective viral replication [4–6]. There is evidence that too much of certain viral non-structural proteins are disadvantageous for certain viruses to successfully replicate [6]. To ensure a proper ratio of viral proteins, some positive-strand RNA viruses employ the host regulatory mechanisms, for example, the UPS, to degrade excess viral proteins to prevent interference of these proteins with the viral replication. In addition, post-translational modification of some viral proteins mediated by ubiquitin and ubiquitin-like proteins of the UPS has also been demonstrated

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Abbreviations used

TMV, tobacco mosaic virus; TYMV, turnip yellow mosaic virus; TBSV, tomato bushy stunt virus; HAV, hepatitis A virus; HCV, hepatitis C virus; EMCV, encephalomyocarditis virus; SARS-CoV, severe acute respiratory syndrome coronavirus; RdRp, RNA-dependent RNA polymerase; NSP4, non-structural protein 4; NS5B, non-structural 5B protein; NS2, non-structural 2 protein 2; E protein, envelope protein; N protein, nucleocapsid protein; MP, movement protein; CP, coat protein.

as an important means to regulate viral protein function without altering protein stability [2].

The objective of this review is to present an overview of current knowledge on how positive-strand RNA viruses interact and subvert the host UPS, a central component of the host protein degradation system, to maintain optimal levels of viral proteins and to modify the functions of virus-encoded proteins. The possibility that UPS-mediated viral protein degradation may also constitute a host defense process against some positive-stranded RNA viral infections will be briefly discussed as well. How positive-stranded RNA viruses utilize the UPS to modify the level/function of host proteins to generate a favorable environment for their infection will not be the focus of the current review (please refer to other reviews on this topic [2,7,8]).

THE UBIQUITIN-PROTEASOME SYSTEM

Ubiquitin-dependent and ubiquitin-independent proteasomal degradation

In eukaryotic cells, the best-known function of the UPS is to degrade misfolded/damaged proteins or intracellular regulatory proteins that are involved in a variety of cellular activities, including cell-cycle regulation, membrane protein trafficking, transcription, antigen presentation, and signal transduction [9,10]. Protein degradation via the UPS starts with the covalent attachment of ubiquitin to a target protein (a process referred to as ubiquitylation) (Figure 1). This process consists of multi-step ATP-dependent enzyme reactions. First, the ubiquitin is activated by ubiquitin-activating enzyme E1 to form an E1-ubiquitin thioester intermediate.

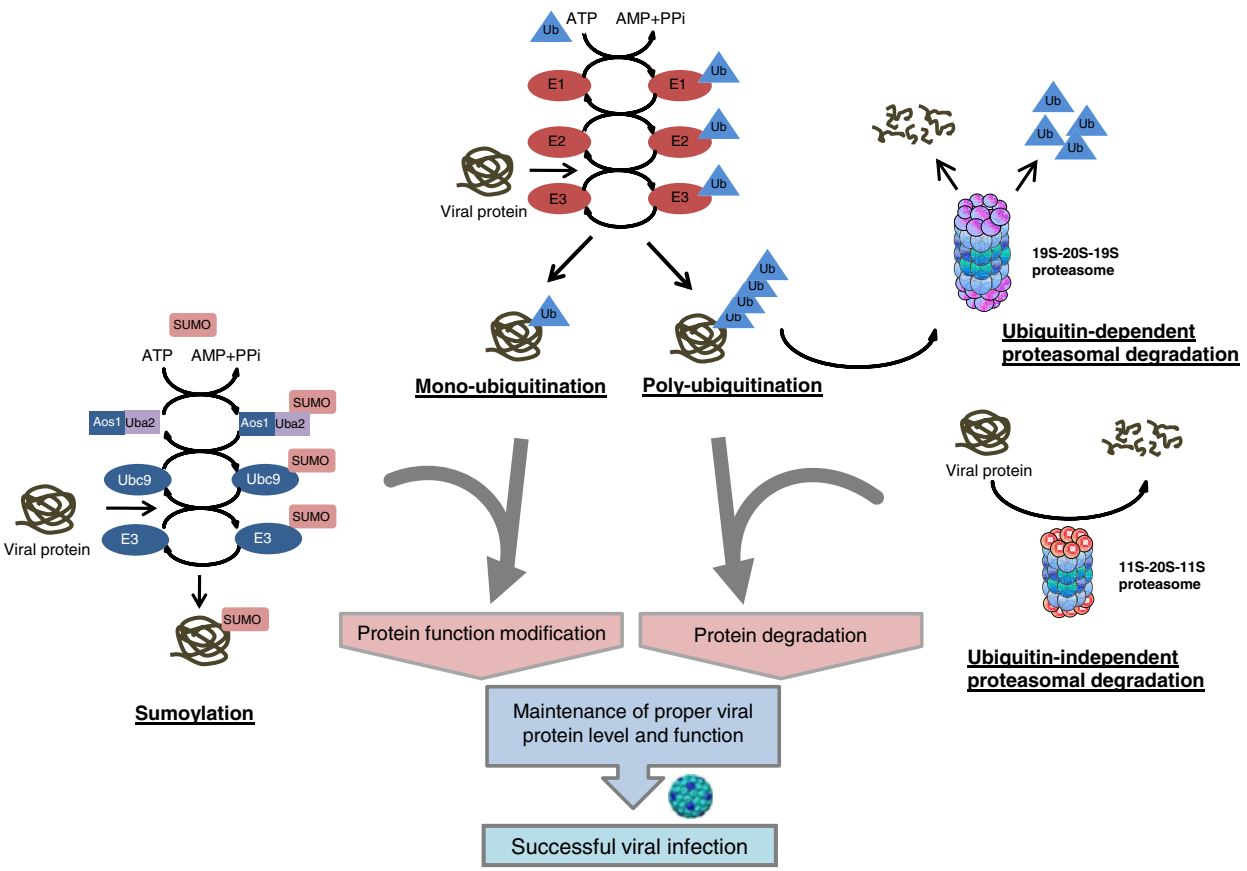

Figure 1. Schematic illustration of post-translational modification of viral proteins by the ubiquitin-proteasome system. Post-translational modification mediated by mono-ubiquitylation or sumoylation is necessary for the regulation of viral protein functions. Proteasomal degradation mediated through either ubiquitin-dependent or ubiquitin-independent mechanisms controls the expression levels of viral proteins. The harmony of these modifications is required to maintain the appropriate level and function of viral proteins, which are crucial for successful infection

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The activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme E2 that subsequently transfers the ubiquitin to the target protein either directly or with the help of a ubiquitin ligase enzyme E3 by forming an isopeptide bond between the carboxyl terminus of ubiquitin and the ε-amino group of a lysine residue on the target protein [11]. The lysine residue of the conjugated ubiquitin is attached by another ubiquitin, consecutively, resulting in the formation of a poly-ubiquitin chain. The ubiquitin-tagged substrate protein is then delivered to the 20S proteasome, and the polypeptide is hydrolyzed into short oligopeptides and the free ubiquitin is recycled through the action of de-ubiquitinating enzymes [12,13]. De-ubiquitinating enzyme plays important roles in UPS as it proofreads ubiquitin-protein conjugates, processes inactive ubiquitin precursors, and keeps sustainable proteolysis by maintaining sufficient ubiquitin within the cell [14]. The 20S proteasome consists of two 7 subunit-composed outer α-rings and two inner β-rings. The function of the α-ring is to translocate the target protein to the core of the β-rings. The β-rings possess the catalytic property to conduct the action of the degradation. The 20S proteasome is normally inactive and inaccessible to protein substrates. Proteasome activators (PA), such as PA700 (also known as 19S proteasome), PA28 (also known as REG or 11S proteasome), and PA200, bind and activate the 20S proteasome [15]. Although the function of PA200 remains to be fully characterized, PA700 has been shown to mainly mediate ubiquitin-dependent proteasomal degradation, whereas PA28 facilitates substrate degradation in a ubiquitin and ATP-independent manner [15] (Figure 1).

Protein modification by ubiquitin and ubiquitin-like proteins

Unlike protein modification by poly-ubiquitylation that mostly influences the stability of substrate protein, attachment of a single ubiquitin, a process called mono-ubiquitylation, regulates the function of target proteins without targeting them for degradation (Figure 1). Mono-ubiquitylation has been shown to alter protein sub-cellular localization, regulate transcriptional and enzymatic activities, and change binding affinities to their partners [16,17].

In addition to ubiquitin, several ubiquitin-like (UBL) proteins have been found to also function as protein modifiers to regulate a variety of cellular functions, including transcription, DNA repair, signal transduction, and cell cycle control, by post-translational modification of target proteins. The best-characterized family of UBL proteins is the small ubiquitin-like modifiers (SUMO), including SUMO-1, SUMO-2, and SUMO-3 [18,19]. Protein modification by sumoylation is directed by an enzymatic cascade parallel to that involved in ubiquitylation (Figure 1). SUMO is activated in an ATP-dependent manner by an E1-activating enzyme consisting of a heterodimer of the Aos1 and Uba2 proteins. After activation, SUMO is transferred to the SUMO E2-conjugating enzyme Ubc9. Three classes of SUMO E3-ligases have been reported, RanBP2, PIAS and the Polycomb protein Pc2. The sumoylation target is a lysine that occurs in the consensus motif ΨKXE where Ψ is a hydrophobic amino acid and X is any residue. Like mono-ubiquitylation, sumoylation does not target proteins for degradation, but instead, regulates protein function and sub-cellular localization [20,21]. A growing body of evidence suggests that sumoylation is particularly important in the regulation of transcription [22]. The role of sumoylation in controlling the function of viral proteins by post-translational modification has also been increasingly recognized [18,23].

Another well-studied UBL molecule is interferon-stimulated gene 15 (*ISG15*). Expression of ISG15 is highly induced upon viral infection and interferon stimulation [24]. Protein modification by ISG15, termed ISGylation, occurs in a mode similar to ubiquitylation and sumoylation that requires the sequential action of E1 (UBE1L), E2 (UbcH8), and E3 enzymes [24]. The process of ISG15 conjugation can be reversed by de-ISGylating enzymes [24]. Function of ISGylation has not been fully understood, but it is thought to alter the biological activities of the target proteins or compete for ubiquitylation and play significant roles in many cellular functions [25]. Although ISGylation has been mainly associated with host defense response against infection of many viruses, including dengue virus [26], West Nile virus [26], Sindbis virus [27], and Japanese encephalitis virus [28], there were reports that the host ISG15 conjugations system can also be exploited by HCV to facilitate viral production [29–31]. The exact mechanism for the

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pro-viral function of ISGylation remains unclear; however, it is speculated that ISGylation of HCV proteins or host proteins critical for HCV life-cycle promotes HCV production by altering the function or preventing target proteins from ubiquitin-mediated degradation [31].

RNA-DEPENDENT RNA POLYMERASE

RNA-dependent RNA polymerase (RdRp) catalyzes the synthesis of new viral RNA genomes from the original viral RNA [32]. Appropriate balance of viral RdRp concentration in infected cells was found to be a key factor affecting the membrane rearrangements, RNA replication, and RNA recombination efficiencies [33–35]. Transgenic overexpression of the 3D RdRp of the Theiler's murine encephalomyelitis virus has been shown to have antiviral effects against the infection of this virus itself and other viruses [6,36]. Although the detailed mechanisms remain to be elucidated, changes of the stoichiometry have been proposed to be one mechanism responsible for such antiviral properties of overexpressed 3D [37]. These studies imply that maintaining an appropriate level of RdRp is important for viral growth [6]. Indeed, the expression levels of RdRp of Sindbis virus, TYMV, HCV, and HAV have been reported to be regulated via UPS-regulated protein proteolysis [5,19,38–40]. It was shown that the RNA polymerase (NSP4) of Sindbis virus is degraded by the proteasome through the ubiquitin-dependent N-end rule pathway [38]. The N-end rule is a highly conserved degradation mechanism relating the stability of a protein with the nature of its N-terminal residue [41]. TYMV RdRp was also shown to be degraded by the proteasome at a late stage of viral infection [5]. This UPS-mediated TYMV RdRp degradation requires prerequisite phosphorylation at two residues (threonine-64 and serine-80) localized within the putative N-terminal PEST sequence (P, proline; E, glutamic acid; S, serine; T, threonine), a known protein degradation motif [19,42].

The RdRps of HAV and HCV are another two examples of UPS-mediated degradation [40]. The HAV 3D polymerase and its precursors were observed to be present at low levels in infected cells [43] and was demonstrated to be poly-ubiquitinated for rapid proteasomal degradation [40]. Nonstructural 5B (NS5B) protein, the RdRp of HCV, was reported to bind to a cellular ubiquitin-like protein [39]. Overexpression of this protein, but not mutant lacking the NS5B-binding domain, increases the poly-ubiquitylation of NS5B and significantly decreases its stability [39].

What is the significance of RdRp degradation during viral infection? Low amounts of RdRp may be required for regulating the switch between the negative-strand and the positive-strand viral RNA synthesis during viral replication [5]. It is also possible that maintaining a low concentration of RdRp is a strategy for virus to preserve its genome integrity [5]. Furthermore, it is conceivable that keeping a low quantity of viral proteins may be beneficial for virus to escape host immune surveillance.

As discussed earlier, in addition to UPS-mediated rapid turnover of viral proteins, viral protein modification by ubiquitin and/or UBLs also plays a key role in the regulation of viral protein function. It has been recently shown that coxsackieviral 3D RdRp can be post-translationally modified by mono-ubiquitylation [44]. Although the specific E3 ligase responsible for 3D ubiquitylation and the exact ubiquitylation site remain undetermined, this modification appears to be required for its function in regulating transcription of viral genome [44]. Besides ubiquitylation, it has also been found that the 3D polymerase of coxsackievirus can be sumoylated with SUMO-1, SUMO-2, and SUMO-3 (unpublished data). DNA alignment reveals one highly conserved sequence Valine-Lysine-Aspartic acid-Glutamic acid (VKDE), which could be found in the 3D of several different species of enteroviral 3D polymerases, that matches the ΨKXE consensus motif for sumoylation, suggesting that sumoylation may also be required for the regulation of 3D transcriptional activity.

AUTOPROTEASE (VIRUS-ENCODED AUTOCATALYTIC PROTEASE)

Autoproteases are virus-encoded proteases that not only proteolytically process viral polyprotein to yield individual structural and non-structural proteins but also mediate the cleavage of many host proteins essential for transcription/translation and maintenance of cellular structures [45,46]. The levels of mature 3C protease of EMCV and HAV, both belonging to the picornavirus family, have been found to decrease rapidly after they reach maximum proteolytic activity, about halfway through the infectious cycle [47–50]. It was later demonstrated that low concentrations of 3C in infected cells are largely attributed to increased protein turnover through ubiquitin-mediated proteasome degradation [48,51–53]. In the presence of

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proteasome inhibitors, poly-ubiquitinated 3C protein accumulated and the rate of 3C degradation was significantly reduced [48,52]. Evidence also suggests that EMCV and HAV exploit the same ubiquitin enzymes for 3C ubiquitylation as 3C proteases of these two viruses were shown to compete with each other for ubiquitin conjugation [48]. Further investigation identified the amino acid sequences ^{34}LLVRGRTLVV^{43} and ^{32}LGVKDDWLLV^{41} that serve as the protein destruction signals for recognition and ubiquitylation of EMCV and HAV 3C proteases, respectively, by the host ubiquitin-conjugating system [40,51–53]. Mutations within these sequences lead to increased stability of 3C proteases. The E3 ubiquitin ligase, E3α, has been shown to recognize these destruction signals of EMCV and HAV 3C proteases and catalyze the conjugation of ubiquitin to them [40,51,53]. The 3C protein of another virus in the picornavirus family, enterovirus 71 (EV71) has also been reported to be regulated by ubiquitin-mediated degradation [54]. It was found that sumoylation of EV71 3C at lysine 52 promotes its ubiquitylation and subsequent degradation [54].

Virus-encoded proteases are required for successful virus replication via cleaving viral polyprotein precursors and can trigger host cell apoptosis by activating pro-apoptotic mediators and suppressing host protein translation and transcription [55,56]. Apoptosis at late stages of viral replication can promote viral progeny release. But premature cell death will perturb viral replication before the virus has completed its life cycle. It is therefore speculated that keeping a small amount of 3C protease is necessary, at least at the early stage of viral infection, to prevent premature cell death and allow efficient viral replication. However, the potential significance of UPS targeting viral protease for degradation in host antiviral defense cannot be completely ruled out.

In the study of the regulation of HCV proteases, it was found that both enzymatically inactive non-structural 2/3 (NS2/3) protein and cleaved NS2 protein of HCV are rapidly degraded during the course of viral infection, underlining the importance of tight regulation of these proteins during the viral life cycle [57–59]. The degradation of NS2 was shown to be regulated in a phosphorylation-dependent manner mediated by casein kinase 2 on serine 164 residue [57]. Notably, ubiquitylation appears not to be required for NS2 degradation as NS2 lysine-to-arginine mutagenesis does not affect its stability and expression levels [57,59]. A non-proteasomal degradation pathway, for example, lysosome-mediated proteolysis, has also been suggested to be involved in the rapid turnover of NS2 [59].

Not all proteases in the family of positive-stranded RNA viruses are regulated by the UPS. The 3C proteases of rhinovirus and poliovirus, similar to those of EMCV and HAV, are present in low concentrations in infected cell [35,60,61]. However, it was found that poliovirus 3C is not conjugated with ubiquitin and remains stable during the viral life cycle [48]. Further studies are warranted to elucidate the mechanisms involved in maintaining low levels of polioviral 3C protease. Possible mechanisms may include blockage of ribosomal read-through and differential processing of polyproteins, leading to reduced production of 3C protease. A recent report has suggested that pseudoknot structures of the SARS-CoV RNA can stop the translation from upstream to downstream encoded viral proteins [62].

Some viral proteases have been shown to possess de-ubiquitinating enzyme activity. It was reported that the papain-like cysteine proteases of coronavirus [63–68], hepatitis E virus [69], and foot-and-mouth disease virus [70] have structural similarity to the cellular de-ubiquitinating enzymes and are able to efficiently hydrolyze ubiquitinated substrates. Although the viral and cellular targets remain largely unknown, the de-ubiquitinating activity of these proteases appears to play a significant role in viral life cycle and in blockage of host innate immunity.

STRUCTURAL PROTEIN

Although the structural proteins need to be expressed at higher levels than non-structural proteins [71], the levels and functions of some viral structural proteins can also be regulated by the UPS. The core protein of HCV is a structural protein that not only packages the viral genomic RNA but also modulates multiple cellular functions, including apoptosis, cell proliferation, cell transformation, and signal transduction, and contributes to HCV pathogenesis [72,73]. The stability of HCV core protein has been reported to be regulated by the proteasome in both ubiquitin-dependent and ubiquitin-independent manner [74–80]. It was demonstrated that the E3 ligase, E6-associated protein (E6AP), binds to the core protein of HCV and promotes its ubiquitylation and subsequent degradation by the

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proteasome [78]. Ubiquitin-independent proteasomal degradation mediated by PA28γ has also been shown to facilitate the degradation of HCV core protein [76,79]. PA28γ interacts directly with the core protein of HCV in the nucleus and regulates its degradation [76]. In animal experiments, this interaction has been demonstrated to play an important role in HCV pathogenesis as PA28γ knockout disrupts the progression of steatosis, hepatocarcinoma, and insulin resistance induced by HCV core protein [74,75]. Moreover, recent evidence revealed a role for PA28γ-mediated and E6AP-mediated degradation of HCV core protein in the regulation of HCV propagation [77].

Recently discovered F protein, a frame-shift product of the core protein of HCV, has also been reported to be directly degraded by the 20S proteasome through a mechanism independent of ubiquitin [81,82]. The function of HCV F protein remains unclear. Available data suggest a role for F protein in HCV pathogenesis by regulating the expression and activity of several pro-inflammatory cytokines, transcriptional factors, and oncogenes [5,83,84]. HCV F protein was found to be very labile with a short half-life (~10 min) and degraded by the proteasome [81,85]. Further experimentation revealed that the degradation of F protein is ubiquitin-independent as a lysine-less F protein mutant remains unstable and its protein levels do not appears to be affected in a cell line with a temperature-sensitive E1 [82]. It was demonstrated that HCV F protein binds to the α3 subunit of the 20S proteasome and is degraded directly by the 20S proteasome *in vitro* [82]. The functional significance of F protein degradation in viral replication (pro-viral strategy versus host defense mechanism) warrants future investigation.

West Nile virus capsid protein is another example of a viral structural protein being the target of the UPS [86,87]. The Makorin ring finger protein 1, a member of the Makorin family of proteins, was identified as the E3 ligase facilitating the ubiquitylation and consequent degradation of this capsid protein by the proteasome [86]. In addition to its role in nucleocapsid assembly, the West Nile capsid protein is also involved in viral pathogenesis by inducing apoptosis [88,89]. Overexpression of Makorin ring finger protein 1 has been shown to result in reduced apoptosis triggered by West Nile virus infection, whereas depletion of this protein promotes viral cytotoxicity [86]. It remains unclear whether such modification and degradation of the capsid protein is a host defense mechanism or a viral strategy to prevent premature cell death.

The envelope (E) protein of SARS-CoV has been reported to be ubiquitinated *in vitro* and in cells, likely through its interaction with the N-terminal ubiquitin-like domain-1 of non-structural protein (NSP3) [90]. The coat protein of TMV has also been shown to be modified by mono-ubiquitylation [91]. However, the functional consequence of these post-translational modifications in the regulation of the viral life cycle and viral pathogenesis is not known and requires further investigation.

Besides ubiquitylation, UBL-mediated modification has also been reported for viral structural proteins. The nucleocapsid (N) protein, a structural protein of SARS-CoV, was demonstrated to undergo post-translational modification by sumoylation [92,93]. Using a yeast two-hybrid system, Ubc9, the E2-conjugating enzyme for sumoylation, was identified as the cellular protein interacting with the SARS-CoV N protein [92]. It was further demonstrated that N protein is covalently modified mainly at lysine 62 residue by SUMO [94]. Further investigation using wild-type N protein and a sumoylation mutant revealed that sumoylation of this protein increases its homo-oligomerization that may play a role in controlling viral replication cycle [94]. Swine fever virus core protein was also found to interact with the intracellular sumoylation pathway [95]. It binds to Ubc9 and SUMO-1 and disruption of these interactions results in attenuated viral virulence, suggesting a regulatory role of SUMO modification in viral infectivity [95]. Dengue virus is a member of the virus family Flaviviridae. Its envelope protein that is responsible for the virus attachment and entry to host cells has been shown to interact directly with SUMO E2 enzyme Ubc9. Further investigation demonstrates that overexpression of Ubc9 reduces the production of infectious virus, suggesting a role for SUMO modification in attenuating viral infectivity [96].

OTHER PROTEINS

Movement protein (MP) is a non-structural protein encoded by plant viruses to facilitate cell to cell movement. TMV MP was previously observed to be only transiently expressed during virus infection [97]. Further investigation demonstrates that TMV MP is poly-ubiquitinated and subsequently degraded by the proteasome and inhibition

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Table 1. Degradation and functional modification of positive-strand RNA viral proteins by the host ubiquitin-proteasome system

Family	Virus	Target protein	UPS-mediated modification	Function (confirmed or proposed)	References
Picornaviruses	HAV	RdRp(3D)	Poly-ubiquitination and degradation	Regulate viral RNA synthesis	Losick *et al.* [40]
		3C protease	Poly-ubiquitination and degradation	Prevent premature cell death	Gladding *et al.* [48]
					Lawson *et al.* [53]
					Losick *et al.* [40]
	EMCV	3C protease	Poly-ubiquitination and degradation	Prevent premature cell death	Lawson *et al.* [51]
					Lawson *et al.* [52]
					Lawson *et al.* [53]
	Enterovirus	3C protease	Poly-ubiquitination and degradation	Prevent premature cell death	Chen *et al.* [54]
			Sumoylation	Promote poly-ubiquitination of 3C	Chen *et al.* [54]
	Coxsackivirus	RdRp (3D)	Mono-ubiquitination	Modulate 3D polymerase function	Si *et al.* [44]
			Sumoylation	Modulate 3D polymerase function	[unpublished]
Flaviviruses	HCV	RdRp (NS5B)	Poly-ubiquitination and degradation	Regulate viral RNA synthesis	Gao *et al.* [2]
		NS2 protease	Ubiquitin-independent degradation		
			Non-proteasomal degradation	Prevent premature cell death	Franck *et al.* [57]
					Welbourn *et al.* [59]
		Core protein	Ubiquitination and degradation	Regulate viral propagation	Moriishi *et al.* [75]
					Shirakura *et al.* [78]
			Ubiquitin-independent degradation	Regulate viral propagation and HCV pathogenesis	Miyamoto *et al.* [74]
					Moriishi *et al.* [75]

Continues

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Table 1. (Continued)

Family	Virus	Target protein	UPS-mediated modification	Function (confirmed or proposed)	References
					Moriishi *et al.* [76]
					Moriishi *et al.* [77]
					Suzuki *et al.* [79]
		F protein	Ubiquitin-independent degradation	Unknown	Xu *et al.* [81]
					Yuksek *et al.* [82]
	West Nile virus	Capsid protein	Poly-ubiquitination and degradation	Prevent premature cell death	Ko *et al.* [86]
					Oh *et al.* [87]
	Swine fever virus	Core protein	Sumoylation	Promote viral propagation	Gladue *et al.* [95]
	Dengue virus	E protein	Sumoylation	Attenuate viral infectivity	Chiu *et al.* [96]
Togaviruses	Sindbis Virus	RdRp (NSP4)	Poly-ubiquitination and degradation	Regulate viral RNA synthesis	de Groot *et al.* [38]
Tymoviruses	TYMV	RdRp (3D)	Poly-ubiquitination and degradation	Regulate viral RNA synthesis	Camborde *et al.* [5]
					Hericourt *et al.* [19]
Tobamoviruses TMV		MP	Poly-ubiquitination and degradation	Regulate viral spread	Reichel *et al.* [98]
		CP	Mono-ubiquitination	Unknown	Dunigan *et al.* [91]
Tombusvirus	TBSV	p33	Mono-ubiquitination	Enhance p33 binding activity	Li *et al.* [99]
				Promote viral replication	Barajas *et al.* [100]
Coronaviruses	SARS-CoV	E Protein	Poly-ubiquitination and degradation	Unknown	Alvarez *et al.* [90]
		N protein	Sumoylation	Promote its homo-oligomerization	Fan *et al.* [92]
					Li *et al.* [93]
					Li *et al.* [94]

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of proteasome function leads to accumulation of MP preferentially on the perinuclear ER [98]. The exact role of UPS-mediated degradation of MP in TMV infection remains to be elucidated. It is speculated that such modification of MP plays a critical role in regulating virus spread by attenuating its damage on ER structure [98].

Ubiquitin-conjugating enzyme Cdc34p has been identified as one of the host proteins binding to the p33 replication protein of Tombusvirus [99]. It was shown that p33 can be ubiquitinated both *in vitro* and *in vivo*, and overexpression of Cdc34p increases, whereas down-regulation of Cdc34p reduces Tombusvirus replication [99]. Further investigation identified the lysine residues required for p33 mono-ubiquitylation and demonstrated the functional significance of p33 ubiquitylation in Tombusvirus replication [100].

The interaction between the host UPS and positive-stranded RNA viruses is summarized in Table 1.

CONCLUSION

Current evidence, reviewed herein, strongly supports a notion that the maintenance of appropriate levels of certain viral proteins within infected cells is crucial for successful viral reproduction. Autoproteases at high concentrations have been suggested to trigger apoptosis of the host cells by cleaving cellular proteins [55,56], whereas high amounts of RdRps have been shown to perturb the process of appropriate viral packaging and even become antiviral when expressed at high levels [6,36]. Growing studies have suggested that positive-stranded RNA viruses manipulate the host UPS for the degradation of excess viral proteins that disturb efficient viral growth and for the modulation of viral protein function through ubiquitin-mediated or UBL-mediated modification (Table 1 and Figure 1). These findings will not only be able to explain the differential expression of various viral proteins but also provide a drug target through a thorough understanding of the mechanism regulating the levels and activities of viral proteins.

CONFLICT OF INTEREST

The authors have no competing interest.

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