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Review

Emerging picture of host chaperone and cyclophilin roles in RNA virus replication

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ABSTRACT

Many plus-strand (+)RNA viruses co-opt protein chaperones from the host cell to assist the synthesis, localization and folding of abundant viral proteins, to regulate viral replication via activation of replication proteins and to interfere with host antiviral responses. The most frequently subverted host chaperones are heat shock protein 70 (Hsp70), Hsp90 and the J-domain co-chaperones. The various roles of these host chaperones in RNA virus replication are presented to illustrate the astonishing repertoire of host chaperone functions that are subverted by RNA viruses. This review also discusses the emerging roles of cyclophilins, which are peptidyl–prolyl isomerases with chaperone functions, in replication of selected (+)RNA viruses.

Contents

Introduction

Plus-stranded (+)RNA viruses, the largest group among viruses, greatly depend on the infected hosts in many steps during their infection cycles. For example, in addition to "borrowing" nucleotides, amino acids and host membranes, viruses also recruit host proteins, termed host factors, to facilitate their replication and spread. The host proteins might contribute to translation of viral RNA to produce replication and structural proteins, or are co-opted to assist various steps in replication, including intracellular localization of viral proteins and RNA, and the assembly of the replicase complex (den Boon and Ahlquist, 2010; Miller and Krijnse-Locker, 2008; Nagy, 2008; Nagy and Pogany, 2010; Novoa et al., 2005). Indeed, hijacked

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host proteins are temporal or permanent residents of the viral replicase complex (VRC), the key enzyme in viral RNA replication.

Successful infection cycle of an RNA virus requires the production of large quantities of viral-coded proteins that need cellular chaperones for correct folding. In addition, viruses co-opt host chaperones to regulate viral replication. Accordingly, many viral replication proteins have to be inserted into cellular membranes to become activated, which likely require protein chaperones. Furthermore, viruses interfere with cellular processes, such as cell signal transduction to avoid premature cell death, via regulating chaperone functions, thus creating favorable microenvironment for their replication. The importance of chaperones in viral RNA multiplication is further supported by the existence of viral RNA-coded chaperone genes in some viral genomes. For example, a viral-encoded Hsp70-like protein is involved in virion assembly and cell-to-cell movement of closteroviruses (Alzhanova et al., 2001; Peremyslov et al., 1999).

This review focuses on how RNA virus infections are affected by cellular Hsp70, Hsp90 and Hsp40, which are central components of the cellular chaperone network. We also discuss the roles of cyclophilins, a different class of cellular chaperones, in replication of some (+)RNA viruses.

Cellular functions of Hsp70 and other protein chaperones

Proteins present in eukaryotic cells are at risk of misfolding and aggregation, which pose a substantial burden to protein homeostasis in cells. Therefore, cells have a large number of protein chaperones and protein remodelling factors that help proteins to obtain their active conformations, mediate refolding and/or degradation of trapped misfolded proteins, facilitate intracellular protein transport, assemble or break up macromolecular complexes (Kampinga and Craig, 2010; Mayer, 2010; Taipale et al., 2010). The most conserved protein chaperones include the ATP-dependent Hsp70 and Hsp90 families; Hsp100; the co-chaperones, including the Hsp40 J-domain proteins (JDP); oligomeric chaperonins (Hsp60s), which form large folding cages to encapsulate protein domains to promote correct folding; as well as the small Hsps, which form oligomeric complexes that bind to denatured proteins in an ATP-independent manner (Kampinga and Craig, 2010; Mayer, 2010; Taipale et al., 2010). There are also specialized chaperones in cells that are involved in highly specific folding of individual proteins or specific complexes, such as the proteosome or the assembly of histones into nucleosomes.

Hsp70 is a highly conserved family of genes in eukaryotes that show high similarity with bacterial DnaK protein chaperone (Ahsen and Pfanner, 1997; James et al., 1997). Hsp70s are involved in a remarkable variety of cellular processes, including the folding of newly synthesized proteins, refolding of misfolded or aggregated proteins, translocation of organellar and secretory proteins across membranes, insertion of membrane proteins into the membrane lipid bilayer, protein complex assembly and disassembly, protein degradation, and receptor signalling (Hartl and Hayer-Hartl, 2002; Wang et al., 2004; Mayer and Bukau, 2005). Consistent with the wide variety of processes Hsp70s take part in, and therefore the versatile substrates they need to regulate, is the prediction that most polypeptides are potential Hsp70-substrates and on average every 30–40 residues in a given protein sequence could interact with Hsp70 (Rudiger et al., 1997). This promiscuous protein recognition is possible because the monomeric Hsp70s and the associated JDP co-chaperones bind short linear stretches of hydrophobic residues in protein substrates (Kampinga and Craig, 2010). Hsp70s function by an ATP hydrolysis-driven conformational change that regulates substrate binding and release (Szabo et al., 1994; Bukau and Horwich, 1998). The intrinsic ATP-hydrolysis activity of Hsp70 is weak (Russell et al., 1999), but can be dramatically enhanced by JDP Hsp70 co-chaperones (Russell et al., 1999) due to stimulation of the ATP-hydrolysis activity of Hsp70s through an interaction between the J-domain and Hsp70 (Cajo et al., 2006; Fan et al., 2003; Hennessy et al., 2005; Qiu et al., 2006). In addition to stimulating ATP-hydrolysis of Hsp70, JDPs also mediate delivery and release of client proteins to Hsp70 (Summers et al., 2009).

JDPs are ubiquitous and comprise a fairly abundant group of Hsp70 co-chaperones, which function as coupling factors among a set of client proteins and Hsp70 (Kampinga and Craig, 2010). While yeast has 22 J-domain proteins, plants have 90–120 JDPs (Miernyk, 2001), suggesting that the number of JDPs is particularly high in plants in comparison to bacteria, yeast, worm, fly and mouse (Rajan and D'Silva, 2009). The reason plants have such a high number of JDPs is not clear, but it may be related to abiotic stress management required by sessile organisms like plants (Rajan and D'Silva, 2009). Besides JDPs, another protein signature that is commonly involved in Hsp70 interactions is the tetratricopeptide repeat (TPR) (Liu et al., 1999). The TPR motif is found in a wide variety of proteins including Hsp70 and Hsp90 co-chaperones (Chen and Smith, 1998; Blatch and Lassle, 1999). Hop (Sti1 in yeast) is a TPR protein that can function to mediate integration of the Hsp70 and Hsp90 chaperone machineries (Chen and Smith, 1998). Recent studies point to a highly complex and regulated chaperone network, in which the fate of client proteins of this network, is determined by the composition of Hsp70-associated co-factors (Kampinga and Craig, 2010). The chaperone network is involved in 'protein triage', in which proteins undergo either productive folding or degradation (Arndt et al., 2007).

Another major protein chaperone family is Hsp90s, which are highly expressed and display ATP-dependent folding function (Taipale et al., 2010). Unlike Hsp70s, which have promiscuous substrates, Hsp90s have a specific set of client proteins, including ~200 signalling proteins, cell-signalling kinases and steroid receptors. However, the function of Hsp90 is dependent on the formation of multiprotein complexes containing other chaperones, such as Hsp70 Hsp104, Hsp40, Hop, immunophilins and others (Taipale et al., 2010).

Up-regulation of Hsp70 production during RNA virus infections

Generation of large amount of viral proteins during infections frequently leads to cellular stress and the up-regulation of Hsps' expression. This is demonstrated by a detailed proteomics analysis of host proteins up- or down-regulated during West Nile virus (WNV), a flavivirus, infection (Pastorino et al., 2009). The authors found that the various isoforms of Hsp70s were up-regulated, while Hsp90s were down-regulated. The increased Hsp70 level could be helpful in protecting the cells from premature apoptosis. Altogether, 13% of the 127 differentially expressed proteins in WNV infected cells are known to have chaperone activities, suggesting that the infected cells try to cope with virus replication with the help of chaperones at the early stage of infection (Pastorino et al., 2009). It is also plausible that WNV could gain easier access to those host chaperones, which are up-regulated during infection, for subverting them to facilitate the viral replication and other viral processes.

Plants infected by various plant viruses express cytosolic Hsp70 proteins at elevated levels, indicating that Hsp70 could play an important role during viral infections (Aparicio et al., 2005; Aranda et al., 1996; Whitham et al., 2003, 2006) (Chen et al., 2008). Interestingly, the increased level of Hsp70 transcription was observed at the front of potyvirus infection, not throughout the entire leaf (Aranda et al., 1996). Recently, transcriptional studies in *Arabidopsis* infected with a potyvirus have shown that numerous Hsps were induced in systemically infected leaves, including Hsp70s (Babu et al., 2008). The induction of Hsp70 might not be directly linked to potyvirus RNA replication since strong ectopic expression of individual potyviral proteins and a variety host proteins are able to induce Hsp70 via a cytoplasmic unfolded protein response (UPR) (Aparicio et al., 2005). Therefore, it appears that Hsp70s are induced as a general response to

the production of high amounts of aggregation-prone viral proteins occurring at later times of infection. Indeed, the induction of Hsp70 correlates with the amount of aggregated viral coat protein (CP) during *Tobacco mosaic virus* (TMV) infection (Jockusch et al., 2001). Even though induction of Hsp70 would be a late response to virus infection, with a role in managing cellular stress and protein homeostasis, this does not exclude the exploitation of abundant Hsp70s by plant viruses to carry out specific tasks relating to virus multiplication as described below.

The role of Hsps in viral RNA replication

In general, Hsps are likely involved in the folding of most viral proteins during and after translation. However, we describe here only the specialized use of Hsps for viral RNA replication (Table 1). One of the best studied cases for co-opting Hsp70 is *Tomato bushy stunt virus* (TBSV), which is a small (+)RNA virus infecting plants. TBSV assembles VRCs on the peroxisomal membrane surface, albeit ER membranes could also serve for this purpose (Jonczyk et al., 2007; McCartney et al., 2005; Panavas et al., 2005; Pathak et al., 2008). Hsp70 is recruited from the cytosol to the site of TBSV replication via its interaction with the p33 and p92^{pol} replication proteins (Figs. 1A–B) (Wang et al., 2009a). Accordingly, proteomics studies revealed that Hsp70 is a permanent resident in the tombusvirus VRC (Serva and Nagy, 2006). Hsp70 likely plays multiple roles during TBSV replication as illustrated schematically in Fig. 1. First, Hsp70 likely affects the intracellular transport/localization of the TBSV replication proteins. Indeed, in the absence of functional Hsp70s in yeast, a model host for TBSV, the p33 and p92^{pol} replication proteins are mislocalized to the cytosol (Fig. 1C) (Wang et al., 2009a, 2009b). Second, functional Hsp70 is needed for the p33 replication protein to bind to the cellular membrane in vitro, suggesting that Hsp70 might facilitate the insertion of the p33 into the lipid bilayer (Figs. 1A–B) (Wang et al., 2009a). Third, Hsp70 also affects the assembly or the activity of the tombusviral VRC, since no active replicase was obtained in an in vitro replicase assembly assay in the absence of Hsp70 (Pogany et al., 2008). The in vitro studies with (i) the yeast cell-free extract and (ii) purified replicase preparations with depleted Hsp70 (Fig. 1D) demonstrated convincingly that Hsp70 is an essential host factor for TBSV replication (Pogany et al., 2008; Wang et al., 2009a). The amount of Hsp70 present in a plant-derived cell-free replication assay correlated with the level of in vitro TBSV replication, suggesting that plant Hsp70s play similar roles as the yeast Hsp70s in supporting TBSV replication (Gursinsky et al., 2009). Importantly, knocking down cytosolic Hsp70 levels by gene silencing or treatment with quercetin inhibitor (Fig. 1E) also reduced TBSV RNA accumulation dramatically in plants (Wang et al., 2009a), supporting the model that Hsp70 is critical for TBSV replication in the native host, too.

Altogether, the data obtained with TBSV have revealed specific and essential roles for a subfamily of cytosolic Hsp70s, represented by four proteins, termed Ssa1–4p in yeast, while direct involvement of other Hsp70s, such as the ribosome-associated Ssb1–2p, Ssc1–2p, the ER resident Kar2p (Bip in mammals), have not yet been found. Albeit the heat-inducible cytosolic Hsps, such as Ssa3p and Ssa4p, can complement the functions of Ssa1p and Ssa2p for TBSV replication in yeast or in the cell-free extract (Pogany et al., 2008; Wang et al., 2009a, 2009b), it seems that Ssa1p and Ssa2p, the two most abundant and constitutively expressed cytosolic Hsp70s, are the favorite Hsps co-opted for TBSV replication.

Systematic targeted analysis of the effect of individual deletion of each protein chaperone gene in yeast on Flock house virus (FHV), a two-component (+)RNA virus infecting insects, revealed that *APJ1* and *YDJ1* Hsp40 genes are needed, while *JJJ1*, *JJJ2* and *ZUO1* were inhibitory on FHV RNA accumulation (Weeks and Miller, 2008; Weeks et al., 2010). These findings suggest complex interactions between FHV replication protein A and various JDPs. Interestingly, the effect of JDPs on FHV and TBSV replication is different, since Jjj1p inhibits FHV RNA accumulation, while over-expression of Jjj1p increases TBSV replication in yeast (Li et al., 2008; Weeks et al., 2010).

A surprising discovery is the involvement of Zuo1p (a JDP), Ssz1p (atypical Hsp70) and Ssb1p/Ssb2p (a typical Hsp70), which can form a heterotrimeric complex with a role in maintaining translation fidelity, in FHV replication (Weeks et al., 2010). This chaperone complex might be important for FHV due to possible difficulties to translate the large FHV protein A with multiple domains. Deletion of any member of the heterotrimeric chaperone complex resulted in increased protein A levels in yeast, suggesting more efficient protein A translation in the absence of these translation fidelity factors (Weeks et al., 2010). The synthesis of FHV protein A is also affected by Hsp90 chaperone (Castorena et al., 2007), but it is currently unknown if the heterotrimeric chaperone complex and Hsp90 function together to regulate the production of protein A.

Co-deletion of *SSA1*/*SSA2* Hsp70 genes reduced FHV replication in yeast (Weeks et al., 2010), similar to the findings observed with TBSV (Serva and Nagy, 2006; Wang et al., 2009a). However, the roles of Hsp70 seem to be different during the replication of FHV and TBSV, since Hsp70 mostly affects FHV protein A accumulation and direct

Table 1
Characterized functions of cellular chaperones in (+)RNA virus multiplication.

Chaperone	Virus	Function in virus multiplication
Hsp70 (Ssa1/2)	TBSV	Localization; component of VRC; membrane insertion and activation of replication proteins
Cyclophilin/parvulin	TBSV	Inhibition of replication, binding to replication proteins
eEF1A	TBSV	Stability and activity of replication proteins
Hsp70 (Ssa1/2)	FHV	Stimulation of replication
Atypical Hsp70s	FHV	Maintaining translation fidelity of protein A
Various JDPs	FHV	Stimulation or inhibition of replication
Hsp72 (Hsp70-like)	HCV	Component of VRC; stability of replication proteins
Hsc70	HCV	Needed for infectivity of HCV particles; assembly of particles on lipid droplets
Hsp90	HCV	Maturation of NS2/3; correct folding of replication proteins; phosphorylation status/stability of NS5A
hB-ind1 (Hsp90-like)	HCV	Correct folding of replication proteins; phosphorylation status/stability of NS5A
FKBP8 immunophilin	HCV	Forms a complex with NS5A and Hsp90, role in replication
CyPA	HCV	Assembly of VRC, folding of NS5A and NS5B
CyPB	HCV	Folding and trafficking of NS5A and NS5B; RNA-binding ability of NS5B
CyPA	Flaviviruses	Possible component of VRC; binds to NS5
Hsp90	Influenza	Stability of polymerase; assembly and nuclear import of the polymerase complex
CyPA	Influenza	Inhibitory, interferes with nuclear localization of M1
Hsp70, CPIP	Potyvirus	Regulation of replication; degradation of coat protein
Ydj1 (Hsp40)	BMV	Assembly of VRC
Hsp60	VSV	Component of viral transcriptase, but missing from VRC
EF-Tu, EFTs	Qbeta	Required for activity of replicase

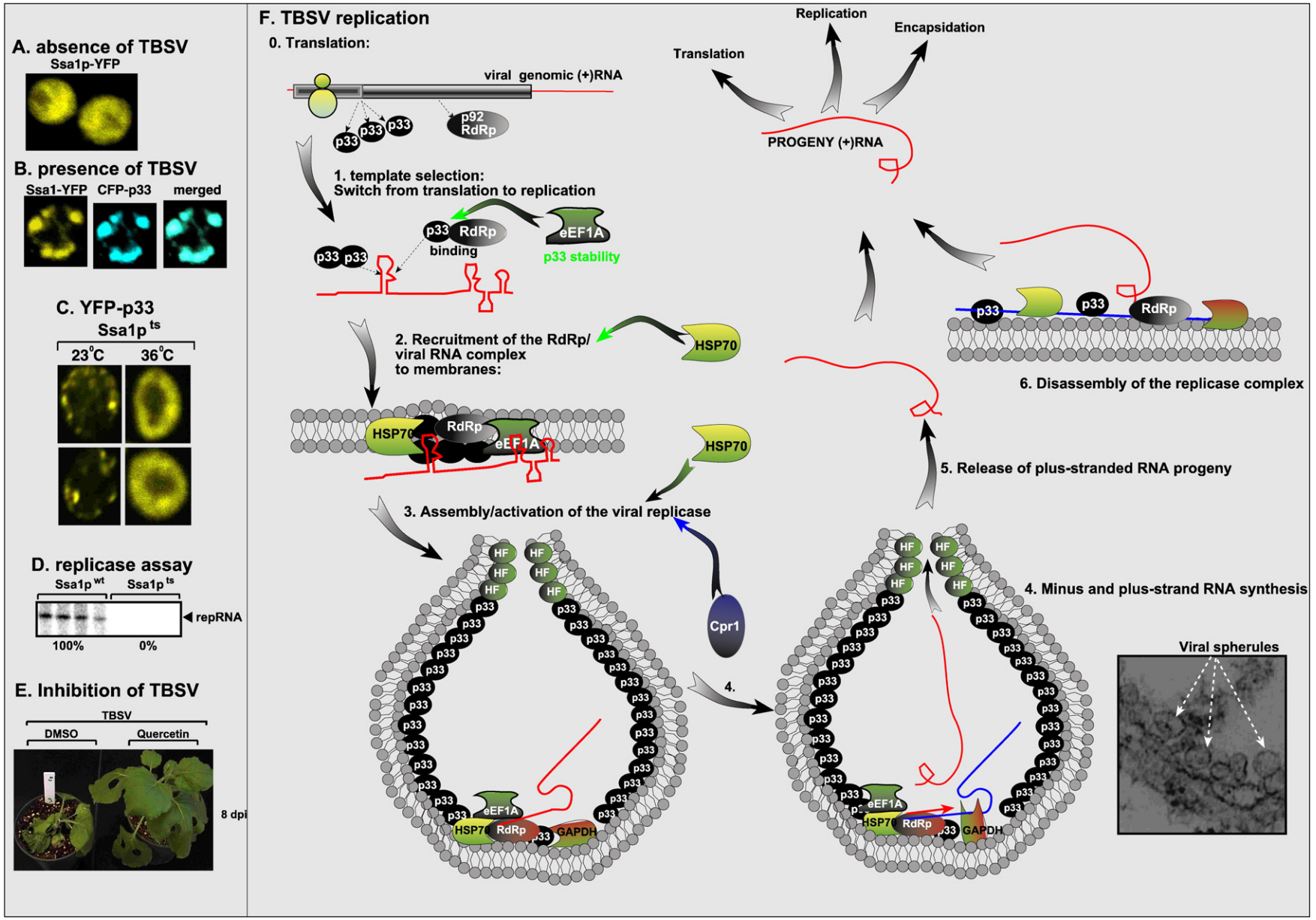

Fig. 1. A model of the roles of host chaperones in TBSV replication. The detailed functions of the host factors are described in the text. (A) Normal cytosolic distribution of Hsp70 (Ssa1p) in yeast cells. (B) Mostly peroxisomal distribution of Hsp70 (Ssa1p) in the presence of replicating TBSV in yeast cells. P33 is a viral replication co-factor, which indicates the sites of VRC. (C) Mostly cytosolic distribution of p33 in yeast cells expressing temperature-sensitive Ssa1p (but lacking Ssa2/3/4) when grown at the nonpremissive 36 °C temperature. (D) Reduced template activity of the tombusvirus replicase when isolated from yeast expressing temperature-sensitive Ssa1p (but lacking Ssa2/3/4) grown at the nonpremissive temperature. (E) Inhibition of TBSV RNA accumulation and symptom formation in *Nicotiana benthamiana* plants treated with quercetin, which reduces Hsp70 levels. (F) Schematic representation of the replication steps and the roles of host chaperones during TBSV replication.

interaction between Hsp70 and Protein A has not been found (Weeks et al., 2010). In contrast, Hsp70 is directly involved and essential for TBSV replication in yeast and in plants (Fig. 1) (Pogany et al., 2008; Wang et al., 2009a, 2009b).

In addition to best-characterized roles of Hsps in TBSV and FHV replication, evidence is growing rapidly that Hsps are also important for the replication of many RNA viruses. Accordingly, Hsp72, a member of the Hsp70 family, was found to interact with NS5A, NS3 and NS5B (RdRp) replication proteins of HCV, suggesting that Hsp72 is part of the HCV VRC (Chen et al., 2010). Down regulation of Hsp72 level led to decreased amount of replicase complexes, while over-expression of Hsp72 increased the amount of replicase complexes likely due to altered stability of the HCV replication proteins or a change in the efficiency of internal ribosome entry (IRES)-based translation of the HCV replication proteins (Chen et al., 2010). Further evidence supporting the role of the Hsp70 family in HCV replication was obtained by using quercetin inhibitor to knock down Hsp70 mRNA transcription that resulted in reduced HCV accumulation and particle production (Gonzalez et al., 2009). Hsp40 co-chaperone was also co-purified with NS5A protein, but the role of Hsp40 has not yet been analyzed in-depth (Gonzalez et al., 2009). Another Hsp70, namely heat shock cognate protein 70 (Hsc70), was also co-purified with NS5A replication protein (Chen et al., 2010), but Hsc70 did not affect HCV replication (Parent et al., 2009). Instead, Hsc70 affected the release of viral particles from cells and infectivity of HCV. Moreover, Hsc70 was present in the viral capsid and co-localized with the HCV core and E2 structural proteins around lipid droplets.

HCV replication also depends on Hsp90 chaperone, which enhances the maturation of NS2/NS3, stability of NS3 as well as forms a complex with HCV NS5A replication protein and the host FKBP8 immunophilin (Okamoto et al., 2006; Ujino et al., 2009). Hsp90 co-chaperone-like human butyrate-induced transcript 1 (hB-ind1), which co-localizes with HCV dsRNA, NS5A and FKBP8 to the membranous web structure induced during HCV replication, interacts with NS5A and likely recruits Hsp90 for HCV replication (Taguwa et al., 2009). Based on the known functions of Hsp90 and co-chaperones, it has been proposed that Hsp90 and hB-ind1 are recruited by HCV for promoting correct folding of the HCV replication proteins, preventing the induction of UPR, and facilitating intracellular movement of HCV-hijacked membranes. Hsp90 and hB-ind1 might also affect the phosphorylation status of NS5A replication protein, which is known to affect the ability of NS5A to support HCV replication (Taguwa et al., 2009).

Hsp70 chaperone family and co-chaperones might also be involved in replication of several (+)RNA and (−)RNA viruses, such as Sindbis virus, *Brome mosaic virus*, *Turnip mosaic virus*, *Tomato mosaic virus* (ToMV), and Borna disease virus, (Brown et al., 2005; Dufresne et al., 2008; Frolova et al., 2006; Hayashi et al., 2009; Nishikiori et al., 2006; Qanungo et al., 2004). For these viruses, Hsp70 and co-chaperones were proposed to stimulate viral RNA-dependent RNA polymerase (RdRp) activity (Momose et al., 2002), help the assembly of the viral replicase or promote replication (Tomita et al., 2003). The polymerase of respiratory syncytial virus (RSV) has been shown to co-localize with HSP70 to lipid-raft membranes and virus-induced inclusion bodies (Brown et al., 2005). Additional, in-depth studies will be needed to address the mechanistic functions of Hsp70s for these viruses.

Replication of other viruses also depends on Hsp90 proteins, including activation of reverse transcriptase for hepadnaviruses (Hu et al., 2004; Stahl et al., 2007; Tavis et al., 1998), the assembly and nuclear import of the influenza A virus RNA polymerase complex and the stability of the L polymerase protein (Connor et al., 2007; Momose et al., 2002; Naito et al., 2007). Most of the above studies highlight Hsp70 and other cellular chaperones as major players during virus replication and virion assembly. It is worth mentioning that Hsp70 and Hsp90 chaperones are also involved in the infectious cycles of DNA viruses by affecting nuclear localization, genome replication and cell transformation, cell entry, virion assembly and disassembly, envelope protein maturation, folding of capsid proteins, and viral transcription (Maggioni and Braakman, 2005; Mayer, 2005).

Unique roles of Hsps in regulation of viral RNA replication

Hsps might also be recruited for virus replication for unique functions, such as regulation of protein composition of VRCs. Indeed, an interesting role of Hsp70 and its J-domain co-chaperone has been unravelled for potyviruses, which are picornavirus-like viruses of plants. Hsp70 has been found to be part of the membrane-bound potyvirus VRC and likely performs functions similar to those needed by other (+)RNA viruses (Dufresne et al., 2008; Hafren et al., 2010). However, Hsp70 has been proposed to have a unique function as well: to regulate the amount of coat protein (CP) present in the VRC at the early stage of replication (Hafren et al., 2010). Since the potyviral CP is produced via cleavage of the polyprotein precursor and the CP is inhibitory to potyvirus replication at the early stage of infection, a JDP, called CPIP, and HSP70 likely function to facilitate the degradation of CP, and thereby preventing CP from causing early cessation of replication (Fig. 2A). Accordingly, down-regulation of Hsp70 caused a CP-mediated defect in viral replication (Hafren et al., 2010). Moreover, the inhibitory effect of over-expression of CP on potyvirus translation and replication could be reduced by co-expression of CPIP, which binds to CP. Interestingly, the host factor CK2 kinase is proposed to affect CP association with CPIP and Hsp70 that leads to ubiquitination of CP and its degradation (Hafren and Makinen, unpublished). At late stage of infection, the increasing amount of CP sequesters CPIP-Hsp70, leading to CP-mediated inhibition of translation and replication and promotion of virion assembly (Fig. 2B) (Hafren et al., 2010). Thus, by co-opting limited amount of chaperones, potyviruses can downregulate CP level during early replication stage, while overwhelming CPIP-Hsp70 by the abundant CP at a later stage, using CPIP-Hsp70 as a regulator of potyvirus multiplication versus virion assembly.

The role of HSPs in viral RNA transcription

Several RNA virus groups produce RNA transcripts that are shorter than the genomic RNA for translation purposes. This strategy is used by (−)RNA viruses to produce viral mRNAs or several (+)RNA viruses to produce subgenomic (sg)RNAs. Hsps have been documented to play a role in viral RNA transcription. For example, the transcriptase complex of vesicular stomatitis virus (VSV) contains Hsp60 in addition to eEF1A, guanylyltransferase and the viral L and P proteins (Qanungo et al., 2004). In contrast, the VSV replicase complex producing the genome-sized RNA progeny lacks the above host proteins, and contains only the viral L, P, and N proteins (Qanungo et al., 2004). The different compositions of the VSV transcriptase and replicase complexes suggest that Hsp60 might be involved in the assembly or the function of the VSV transcriptase. The possible role of Hsps in the assembly of the VSV replicase is currently unknown.

HSPs as targets for antiviral approaches

RNA viruses evolve rapidly and many drugs and vaccines can be rendered useless by the newly emerging resistant viruses. However, targeting host proteins needed for virus multiplication could be an alternative approach for broad and durable resistance against RNA viruses. Accordingly, pharmacological inhibition of Hsp90 impaired the replication of poliovirus, rhinovirus and coxsackievirus in cell culture or in infected animals. Importantly, the anti-Hsp90 treatment did not lead to the emergence of drug-resistant escape mutant viruses (Geller et al., 2007). The use of quercetin as an anti-Hsp70 compound also resulted in reduced accumulation of TBSV, *Tobacco mosaic virus*, *Turnip crinkle virus*, *Potato virus A* and HCV (Gonzalez et al., 2009; Hafren et al., 2010; Wang et al., 2009a). Therefore, targeting host

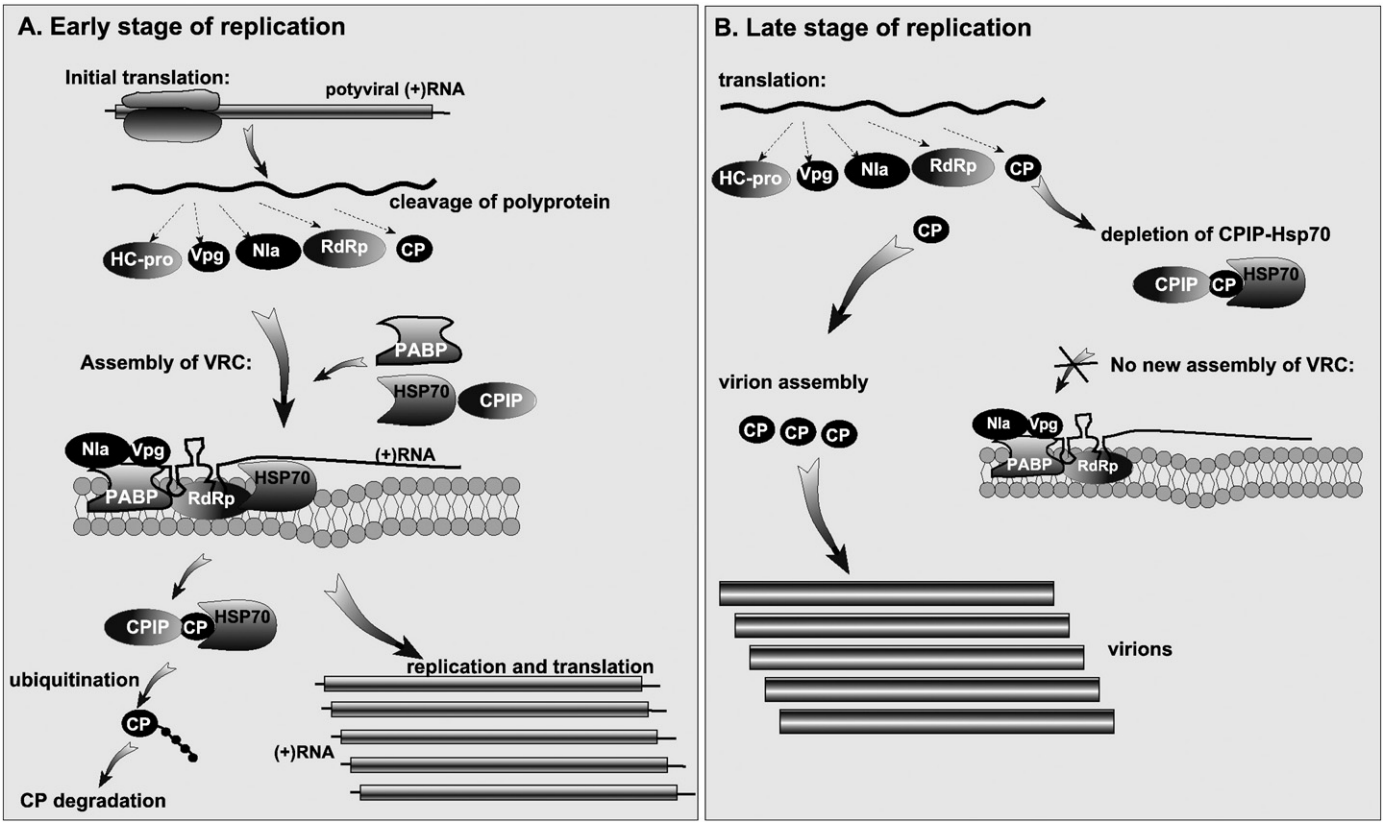

Fig. 2. A model of the roles of Hsp70 and CPIP co-chaperone in potyvirus replication. The detailed functions of the host factors are described in the text. (A) The interaction between CPIP-Hsp70 and the potyvirus coat protein (CP) leads to ubiquitination and degradation of CP. This allows replication and additional translation to occur during the early phase of infection. (B) The increasing amount of CP depletes CPIP-Hsp70, which leads to shut down of replication and translation. Then, the CP will be involved in RNA encapsidation and virion assembly at the late stage of infection.

chaperones involved in viral protein folding might be a general, broad antiviral strategy.

Cellular functions of cyclophilins

Cyclophilins together with the structurally unrelated FKBPs and parvulins are a large family of peptidyl–prolyl cis–trans isomerases (PPIase) that also have protein chaperone-like function (Wang and Heitman, 2005). Isomerization of peptidyl–prolyl bonds is frequently required for protein refolding, maturation and trafficking and PPIases play a global role in facilitating correct protein folding and conformational changes in client proteins (Arevalo-Rodriguez et al., 2004; Wang and Heitman, 2005). Cyclophilins share a common 109 aa cyclophilin-like domain (CLD) and additional domains unique to each member of the family. The CLD domain contains the PPIase activity, while the unique domains are important for selection of protein substrates and subcellular compartmentalization. *S. cerevisiae* and *Arabidopsis* have 8 and 29 cyclophilins, while humans have 16 cyclophilin isoforms that have different cellular and tissue distribution (Arevalo-Rodriguez et al., 2004; Galat, 2004; Galat and Bua, 2010). The most important functions of cyclophilins in cells are in protein folding, assembly of multidomain proteins, muscle differentiation, detoxification of reactive oxygen species, immune response, and in various diseases, such as cancer, atherosclerosis, diabetes and neurodegenerative diseases (Galat and Bua, 2010; Lee and Kim, 2010; Wang and Heitman, 2005).

A critical role for cyclophilins was suggested by the finding that cyclosporine, an inhibitory peptide of cyclophilins, markedly inhibited HCV replication in cell cultures (Nakagawa et al., 2004; Watashi et al., 2003). Follow up experiments suggested the involvement of both cyclophilin A (CyPA) and CyPB in HCV replication (Heck et al., 2009; Kaul et al., 2009; Yang et al., 2008). However, the roles of individual cyclophilins in HCV replication are somewhat inconsistent and contradictory (Chatterji et al., 2009, 2010; Gaither et al., 2010; Kaul et al., 2009; Liu et al., 2009b; Yang et al., 2010). The emerging picture is that HCV replication is affected by multiple cyclophilins. One function of PPIases is to play a role in the assembly of the HCV VRC. Accordingly, the cytosolic CyPA has been shown to be critical for the assembly of the HCV VRC, by likely affecting the processing of the HCV polyprotein, the cleavage of NS5A–NS5B fusion protein, the folding of the NS5A and NS5B RdRp (Coelmont et al., 2010; Kaul et al., 2009). The potential targets of CyPA include NS5B, NS5A and NS2, and these interactions were postulated to play a role in stabilization of the HCV VRC. In addition, CyPA inhibition could affect HCV replication in several other ways, such as inhibition of transcription of hVAP, La autoantigen or other host factors or affecting chemokine levels. Selection of functional, but CyPA-independent, NS5A mutants revealed that CyPA is likely involved in folding of a nonstructured region in NS5A, which might require a structural shift towards a more extended conformation needed for proper function of NS5A during HCV replication (Yang et al., 2010).

Cyclophilins may potentially affect the conformation of the viral RdRp or the RNA template, which in turn could alter the template activity of the RdRp. Indeed, CyPB, a cellular PPIase resident in the ER-Golgi, was found to regulate the RNA-binding ability of the HCV NS5B RdRp (Watashi et al., 2005). In addition, CyPB might affect HCV replication via binding to NS5A and NS5B replication proteins and by promoting viral RNA synthesis, and possibly stimulating protein folding and trafficking. Similarly, it has been proposed that CyPC, which is also ER-Golgi-resident protein, affects the trafficking of the viral proteins (Gaither et al., 2010). siRNA-based mRNA depletion experiments also indicated that additional cyclophilins, CyPH, CyPE

and CyP-40 participate in cellular pathways important for efficient HCV replication, but the mechanisms are not yet studied (Gaither et al., 2010). Based on the significance of cyclophilins in HCV replication, several inhibitors of cyclophilins are currently in clinical trials for HCV therapy.

CyPA seems to affect multiplication of several flaviviruses, such as WNV, Dengue virus and yellow fever virus (Qing et al., 2009). CyPA was shown to bind to the NS5 replication protein and is likely component of the VRC and cyclosporine inhibitor has been shown to inhibit flaviviral RNA synthesis (Qing et al., 2009).

Additional PPIase involved in HCV replication is the human FKBP8 immunophilin, which was shown to interact via the three TPR repeats with the HCV NS5A, a membrane anchored replication protein (Okamoto et al., 2006). siRNA-based knockdown of FKBP8 led to reduced HCV replication in human hepatoma cell line, suggesting that FKBP8 is important for HCV replication. Interestingly, FKBP8 might function in HCV replication by forming a complex consisting of NS5A, FKBP8 and Hsp90 (Okamoto et al., 2006). Interaction between NS5A and FKBPs is also important for HCV to block apoptosis in hepatoma cells (Wang et al., 2006).

In contrast to the stimulatory role during HCV infection, cyclophilins have been shown to inhibit accumulation of several RNA viruses. For example, Cpr1p (the yeast homolog of the mammalian CyPA) interacts with the tombusvirus replication proteins in yeast and in vitro resulting in reduced level of TBSV replication (Mendu et al., 2010). It has been proposed that direct binding between Cpr1p and the TBSV replication proteins blocks p33/p92^{pol} functions or the structural changes in p33/p92^{pol} caused by the prolyl isomerase activity of Cpr1p might be responsible for the inhibitory effect of Cpr1p on TBSV replication. Interestingly, Ess1p parvulin PPIase, whose function can be complemented by over-expression of *CPR1* (Arevalo-Rodriguez et al., 2004), was also found to inhibit TBSV replication (Mendu et al., 2010). These PPIases might complement each other, since yeast strain with deletion of *CPR1* and a temperature-sensitive *ess1* supported increased level of TBSV replication (Mendu et al., 2010).

Another example for the antiviral activity of cyclophilins is shown for influenza A virus. CypA was found to bind to the matrix protein (M1) of influenza A virus and interfered with the nuclear localization of M1 (Liu et al., 2009a). Over-expression of CypA increased the self-association of M1 and led to decreased viral replication.

CypA was also found to inhibit the infectivity of HIV-1 (human immunodeficiency virus-1) virions (Sokolskaja and Luban, 2006; Strebel et al., 2009). Interestingly, a CyPA-TRIM5 fusion protein, emerged as a retrotransposition of *CyPA* between exons 7 and 8 in *TRIM5* gene, in New World owl monkeys targets HIV-1 coat protein causing resistance to HIV-1 infections (Berthoux et al., 2005; Brennan et al., 2008). CypA gets incorporated into HIV-1 virions due to direct binding to Gag, the polyprotein precursor of virion structural proteins (Franke et al., 1994; Luban et al., 1993; Strebel et al., 2009). The role of CypA as an anti-HIV protein is neutralized by the retroviral Vif protein, which inhibits the incorporation of CypA into the viral particles (Takeuchi et al., 2007). Overall, the published data indicate that prolyl isomerases are potent inhibitors of several RNA viruses and they might be part of the innate response of the host against some viruses. Since PPIases are conserved and ubiquitous proteins, their role could be common against viruses.

Noncanonical protein chaperone functions

In addition to the Hsps, additional cellular proteins with chaperone functions are co-opted for RNA virus replication. The best characterized among such chaperones are translation elongation factors EF-Tu (eEF1A in eukaryotes) and EF-Ts that are necessary for replicase assembly of Qbeta bacteriophage (Takeshita and Tomita, 2010). Binding of EF-Tu and EF-Ts to the catalytic subunit is required to maintain the active conformation of the replicase (Takeshita and Tomita, 2010). Interestingly, eEF1A regulates TBSV replication via binding to the viral replication proteins in VRC, suggesting a similar role for the co-opted eEF1A in TBSV replication to the role played by EF-Tu for Qbeta (Li et al., 2009, 2010). Moreover, mutations in eEF1A resulted in rapid degradation of the tombusvirus p33 replication protein, suggesting that eEF1A is needed to maintain the proper folding and stability of the viral replication protein during replication (Li et al., 2009). Since eEF1A was found to affect the replication of many RNA viruses, including WNV, Dengue virus, TMV, *Turnip yellow mosaic virus* (TYMV), *Turnip mosaic virus* (De Nova-Ocampo et al., 2002; Nishikiori et al., 2006; Thivierge et al., 2008; Zeenko et al., 2002) (Dreher, 1999), Bovine viral diarrhea virus (Johnson et al., 2001), HCV (Kou et al., 2006), (Yamaji et al., 2006), HIV-1 (Cimarelli and Luban, 1999) and VSV (Qanungo et al., 2004), the chaperone activity of eEF1A could be exploited by these viruses as well.

Conclusion and future perspectives

Two groups of host factors identified for many RNA viruses are the heat shock chaperone family, including HSP70, the J-domain group, HSP90 and the cyclophilin family. These chaperones affect many aspects of virus multiplication, through regulating translation, replication, virion assembly and counterdefence against cell apoptosis or native immune responses. The subverted chaperones facilitate viral protein folding, activation, subcellular localization, membrane insertions and protein stability. The co-opted Hsps likely play major roles in assembly of the VRC and its activity. In addition to the canonical roles, several Hsps and cyclophilins likely perform unique functions during replication of RNA viruses.

In spite of the recent significant advances, we still do not know the answers to many questions on how the heat shock proteins and co-factors are involved in virus replication. The use of proteomics approaches and structural studies will help define the roles of chaperones in regulating the composition and structure of viral protein complexes, while super-resolution microscopy and electron tomography will define the subcellular compartments and temporal activities of chaperones in viral complexes. Additional cell-free methods will likely dissect the roles of chaperones in regulation of the activities of VRC. The gained knowledge will be used to interfere with the functions of various cellular chaperones to inhibit virus multiplication. The new chaperone-based antiviral approaches could be durable and broad against many viruses.

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