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Biochim Biophys Acta. Author manuscript; available in PMC 2013 March 01.

Published in final edited form as:
Biochim Biophys Acta. 2012 March ; 1823(3): 698–706. doi:10.1016/j.bbamcr.2011.11.007.

Broad action of Hsp90 as a host chaperone required for viral replication

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Abstract

Viruses are intracellular pathogens responsible for a vast number of human diseases. Due to their small genome size, viruses rely primarily on the biosynthetic apparatus of the host for their replication. Recent work has shown that the molecular chaperone Hsp90 is nearly universally required for viral protein homeostasis. As observed for many endogenous cellular proteins, numerous different viral proteins have been shown to require Hsp90 for their folding, assembly, and maturation. Importantly, the unique characteristics of viral replication cause viruses to be hypersensitive to Hsp90 inhibition, thus providing a novel therapeutic avenue for the development of broad-spectrum antiviral drugs. The major developments in this emerging field are hereby discussed.

Keywords

Hsp90; Antivirals; Chaperones; Drug resistance; Hsp90 inhibitors; Virus; protein folding

1. Introduction

The capacity of all proteins to carry out their function is dependent on their ability to fold correctly and folding of many cellular proteins critically relies on the assistance of molecular chaperones. Molecular chaperones are highly conserved and often essential for viability and for the ability to survive cellular stress. Chaperones reside in all cellular compartments where they promote the folding, maturation, complex assembly, and trafficking of proteins in an ATP dependent manner [1-3]. In addition, chaperones provide a conduit to the protein degradation machinery and regulate many cellular pathways [3].

Viruses are intracellular obligate parasites that hijack the biosynthetic machinery of the host for their replication. Given the structural and functional complexity of many viral proteins, it is not surprising that they, like cellular proteins, are also dependent on chaperones for their folding and function. In this review we discuss the role of Hsp90 in viral replication and the potential of targeting Hsp90 as an antiviral strategy.

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2. Hsp90: Overview of cellular functions

Hsp90s are a family of highly abundant, essential, and evolutionary conserved molecular chaperones. In mammals, there are two cytoplasmic Hsp90 isoforms, the stress induced Hsp90α and the constitutively expressed Hsp90β [4], as well as an ER resident homologue Grp94 (also called gp96) [5], and a mitochondrial variant, TRAP1 [6]. Hsp90s are ATPases that mostly form homodimeric complexes, with each monomer sharing a common domain organization comprised of a C terminal dimerization domain, a middle domain, and a structurally unique N terminal ATPase domain (see review [7, 8]). In the cytoplasm, Hsp90 regulates the activity, maturation, localization, and turnover of a large yet select set of substrates or "clients" [9, 10]. Hsp90 function is regulated by a cohort of co-chaperones that modulate its ATPase cycle, enable client acquisition and selection, and provide a link to other chaperone systems as well as the ubiquitin-proteasome system [11-13]. Hsp90 is believed to recognize a metastable structural element in its clients rather than a primary amino acid sequence. Unlike other chaperone systems in the cell, Hsp90 does not appear to interact with newly synthesized proteins. Instead, Hsp90 receives its client proteins from other chaperone systems, such as Cdc37 or the co-translationally acting Hsp70 chaperone system, likely following the acquisition of a partially folded state recognized by Hsp90. Thus, some of the client specificity of Hsp90 is determined by these collaborating chaperones. For protein kinases, which comprise the most intensely studied client group, binding to Hsp90 requires the Cdc37 co-chaperone, which binds to both Hsp90 and protein kinase clients [14]. For other clients, such as steroid hormone receptors, Hsp70 binds first, and the co-chaperone Hop bridges transfer to Hsp90. Hop physically interacts with both chaperone systems by virtue of specific tetratricopeptide (TPR) interaction domains that recognize the C-terminal tails of both Hsp70 and Hsp90 [15, 16]. In addition to its role in assisting the maturation of proteins, Hsp90 was shown to be specifically required for the degradation of certain proteins [17]. In this regard, it is noteworthy that CHIP, another TPR containing protein that binds to Hsp90 also contains a U-box domain with E3 ubiquitin ligase activity. CHIP has been shown to direct Hsp90 and Hsp70 bound proteins to proteasomal degradation [18].

The cellular functions and targets of Hsp90 are highly diverse. In addition to its originally described function assisting the maturation of protein kinases and steroid hormone receptors [19], Hsp90 is required for the function of a large number of proteins and protein complexes, both under normal growth conditions and under conditions of stress, such as elevated temperatures. Hsp90 clients include proteins involved in transcription, translation, mitochondrial function, kinetochore assembly, centrosome function and cell cycle [20-22]. Hsp90 is also central to secretory pathway function and associates with many complexes involved in membrane trafficking [3, 22]. Many of the Hsp90 clients are part of multisubunit complexes. The current understanding of Hsp90 function as a chaperone that stabilizes or remodels polypeptides that are substantially folded suggests that its action promotes a conformational maturation step that enables clients to bind ligands, interact with cofactors, or carry out their biological function [23]. For instance, many clients of Hsp90 undergo ordered assembly and disassembly processes, and Hsp90 may stabilize subunits of these complexes prior to assembly or facilitate their conformational transitions [24]. During cellular stress, e.g. at elevated temperatures, Hsp90 may also become important to stabilize the labile conformations of many proteins, most notably cell cycle components. In this regard, it is noteworthy that aberrant proteins with a few amino acid changes exhibit radically higher dependence on Hsp90 than their native counterparts [25].

Among the Hsp90 clientele identified to date are many proteins involved in signal transduction and cell division, including numerous proteins related to tumorigenesis [26, 27]. The dependence of multiple oncogenes on Hsp90 renders cancer cells hypersensitive to

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Hsp90 inhibition and forms the basis for using Hsp90 inhibitors in cancer treatment. Numerous specific pharmacological inhibitors of Hsp90 have been identified. The majority of these, including radicicol, Geldanamycin (GA), and the GA derivatives 17-allyl-17-demethoxygeldanamycin (17-AAG) and 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin (17-DMAG), inhibit Hsp90 activity by competing with ATP for binding to the N-terminal ATP/ADP-binding domain of Hsp90 (see [28] for review). Inhibitors that bind to the C-terminal domain, such as novobiocin and coumermycin A1, have also been identified. Due to the fact that many Hsp90 clients are involved in cancer progression, these inhibitors possess broad anticancer activity and several are currently undergoing advanced stage clinical evaluation [26, 29].

3. Hsp90 in viral replication

Viral proteins, like cellular proteins, are dependent on chaperone function for folding and assembly [30]. Hsp90 has been shown to play a role in the replication of many different viruses including DNA viruses, RNA viruses of both positive and negative sense genomes, and double-stranded RNA viruses (see Table 1). In fact, the dependence of viruses on Hsp90 appears to be nearly universal. Strikingly, for viruses tested to date, replication appears to be sensitive to Hsp90 inhibitors at concentrations not affecting cellular viability. The hypersensitivity of viral replication to Hsp90 inhibition may stem from several unique characteristics of viral protein homeostasis, which present a distinct set of challenges to the protein folding machinery, and thus render it more sensitive to fluctuations in cellular folding capacity. First, the limited size of most viral genomes often determines that viral proteins be multifunctional, likely resulting in structurally complex proteins that are dependent on chaperone function. In addition, many viruses, and in particular cytopathic viruses, must produce large quantities of a limited number of viral proteins within a short period of time. The need to rapidly produce a limited array of structurally complex proteins in high abundance is likely to tax the capacity of the chaperone systems required to fold them. Moreover, the unique complexity and structural requirements of viral capsids make their precursors particularly vulnerable to aggregation and misfolding [31-34]. Thus, capsid proteins must fold to a precursor conformation that is soluble yet poised to assemble in the presence of the viral genome to form a capsid, which may contain over 1000 identical subunits and retain infectivity despite extreme thermal and chemical conditions [35]. The high mutation rates of RNA viruses, which are the highest in nature, inevitably lead to the production of a swarm of mutant viral proteins during infection. Such viral population diversity has been shown to be critical for the ability of viruses to adapt to adverse conditions [36-38]. However, the mutant proteins thus generated can have dominant negative effects on viral protein complex assemblies [39]. As mutant proteins are known to be hyperdependent on chaperone function for activity [25], chaperones are likely to be instrumental in buffering the deleterious effects of mutant viral proteins and thus facilitate viral adaptation. Finally, since chaperones regulate many cellular functions including signaling networks, cell cycle progression, and apoptosis, viruses are likely to manipulate chaperones to render cells conducive to viral replication.

While the antiviral effects of Hsp90 inhibition have been broadly demonstrated, detailed understanding of the role of Hsp90 in viral replication is only available for a few viruses. In most cases, the antiviral activity of Hsp90 inhibitors is accompanied by the degradation of a single viral protein, implicating such viral proteins as dependent on Hsp90 for their maturation or stability. The major categories of viral Hsp90 clients are outlined below.

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4. Viral Non-Structural Proteins as Hsp90 clients

4.1. Viral Polymerases

Viral polymerases constitute the largest class of identified Hsp90 viral client proteins. The role of Hsp90 in the function of viral polymerases appears to be variable, although detailed knowledge is only available in a few cases. The best-elucidated example of Hsp90's role in polymerase function comes from the reverse transcriptases (RT) of two viruses belonging to the Hepadnaviridae family: duck hepatitis B virus (DHBV) and human hepatitis B virus (HBV). These enzymes mediate both the incorporation of viral pregenomic RNA (pgRNA) into nucleocapsids and the reverse transcription of pgRNA into DNA [40]. For DHBV, Hsp90 stimulates the ability of the RT to initiate and maintain stable reverse transcription. This function of Hsp90 requires the presence of other chaperones, such as Hsp70 and Hsp40, and co-chaperones Hop and p23, which together function as substrate release factors and facilitated incorporation of the pgRNA into nucleocapsids [41-43]. The Hsp90 cofactor Cdc37, normally required for the folding of Hsp90 dependent cellular kinases, was also shown to interact with the DHBV RT independently of Hsp90 [44]. Overexpression of Cdc37 resulted in increased reverse transcription and pgRNA packaging into nucleocapsids, while overexpression of a dominant negative Cdc37 mutant had opposite effects. For HBV, Hsp90 inhibition with GA has been shown to reduce both reverse transcription and pgRNA incorporation into nucleocapsids. Furthermore, the production of active reverse transcriptase *in vitro* requires the addition of recombinant Hsp90, Hsp70, Hsp40, Hop and p23, similar to DHBV [45, 46].

The polymerase of influenza virus A also requires Hsp90 for genome replication (Fig. 1A). The RNA dependent RNA polymerase of influenza virus is comprised of three subunits, PB1, PB2 and PA. Hsp90 associates with both PB1 and PB2 in infected cells and re-localizes to the nucleus where it facilitates viral RNA synthesis [47, 48]. Hsp90 inhibition by treatment with GA or 17-AAG was shown to enhance the degradation of both PB1 and PB2 as well as to reduce levels of the fully assembled polymerase complex, thus reducing the levels of viral derived RNAs [49].

Hsp90 is important for the polymerase functions of Herpesviridae family. The alpha herpesvirus, herpes simplex virus type 1 (HSV-1) induces formation of specific chaperone-rich nuclear structures called “Virus-induce Chaperone enriched” (VICE) compartments, which appear to facilitate viral replication in the nucleus and nuclear protein quality control [50]. During HSV infection, a subpopulation of Hsp90 localizes to VICE compartments and its ATPase activity is required for the proper nuclear localization of viral DNA-dependent DNA polymerase, UL30. Inhibition of Hsp90 with GA results in mislocalization of the viral polymerase to the cytoplasm instead of the nucleus, proteasomal degradation of the polymerase, and consequent reduction of viral DNA replication and production of progeny viral particles [51, 52]. Hsp90 is also required for replication of beta-herpesviruses. Hsp90 inhibition with GA led to degradation of the viral polymerase as well as reduced viral gene expression via the disruption of PI3-kinase pathway during human cytomegalovirus (HCMV) infection [53]. Whether Hsp90 is required for the nuclear localization of the HCMV polymerase, as in the case of HSV1, remains to be tested. On the other hand, during the infection with another alpha-herpesvirus, varicella zoster virus (VZV), the viral single-stranded DNA binding protein ORF29p was shown to colocalize with Hsp90 and Hsp70 in the nucleus through a specific interaction with BAG3 [54]. BAG3 is one of a cohort of Hsp70 interacting Bag-family proteins that modulate Hsp70 function [55, 56]. Hsp90 inhibition by treatment with GA or 17-DMAG resulted in diffuse cytosolic localization of ORF29p and BAG3, suggesting that herpesvirus DNA binding proteins may generally require Hsp90 for nuclear import. BAG3 co-immunoprecipitates with ICP0, the HSV E3 ubiquitin ligase and major pathogenicity factor, but whereas knockdown of BAG3 reduced

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the replication of VZV it did not affect wild type HSV replication [57]. This raises the possibility that viruses utilize different chaperone complexes for the same purpose. It will be of interest to establish whether the polymerases of herpesviruses require Hsp90 for folding and/or nuclear localization directly or whether Hsp90 is required for the folding or activity of cellular factors required for polymerase stability or nuclear import.

Protein A, the RNA dependent RNA polymerase protein of the Nodaviridae flock house virus (FHV), is also dependent on Hsp90. Interestingly in the case of FHV, Hsp90 appears to be required for translation of protein A but not for substantial polymerase activity [58]. Inhibition of Hsp90 with GA during FHV infection results in reduced levels of polysomes translating protein A, while no effect is observed on other viral proteins or cellular RNA associated with polysomes [59]. Whether the role of Hsp90 in protein A synthesis is due to a direct effect on protein A or via the chaperoning of a cellular factor is unknown. In addition to the above examples, other viral families also require Hsp90 for polymerase activity since pharmacological inhibition of Hsp90 by GA during infection results in the degradation of the RNA-dependent RNA polymerases of rhabdoviruses (vesicular stomatitis virus), paramyxoviruses (human parainfluenza 2 and 3, simian virus 5 and 41), and bunyaviruses (La Cross virus), and the concomitant suppression of viral propagation [60].

The dependence of viral polymerases on Hsp90 is very striking as it extends to the RNA-dependent RNA polymerases of both positive and negative-strand RNA viruses, DNA-dependent DNA polymerases, as well as to the reverse transcriptase of hepadnaviruses, despite the evolutionary, structural and functional divergence of these proteins. In this regard, viral polymerases may share features of their cellular counterparts, as telomerase and DNA polymerase α are known Hsp90 client proteins [61, 62]. The general dependence of many polymerases on Hsp90 suggests that this chaperone may recognize a conserved feature of these diverse enzymes, or may facilitate a common step in their maturation and/or mechanism, such as the interaction with nucleic acid. However, not all viral polymerases require Hsp90 for their function [33]. Nonetheless, it will be of interest to elucidate the reason underlying the wide dependence of polymerases function on hsp90.

4.2. Other Non-structural proteins

Hsp90 is also required for the activity and folding of non-structural proteins other than polymerases. In the case of the DNA tumor virus SV40, Hsp90 associates with the large T antigen (LT) [63]. LT protein is a multifunctional protein that interacts with several cellular proteins, including the chaperones Hsp70 and Hsp90, as well as regulatory proteins, such as p53 and DNA polymerase α [64, 65]. Hsp90 physically interacts with LT and Hsp90 inhibition with GA or radicicol leads to its degradation in cells. Similarly, for rotavirus, nonstructural protein 3 (NSP3) also requires Hsp90 for folding and stabilization [66]. NSP3 shuts off cellular translation by relocalizing the cytoplasmic poly(A) binding protein (PABP-C1) to the nucleus, thereby evicting it from translation initiation complexes in infected cells [67-69]. Inhibition of Hsp90 by 17-AAG or 17-DMAG resulted in reduced NSP3 translation and abolishes nuclear translocation of PABP-C1, thus suppressing viral replication [66, 70].

In the case of hepatitis C virus (HCV), Hsp90 is required for the activity and stability of two non-structural proteins, the protease and helicase NS3 and the multifunctional protein NS5A (Fig. 1B). Inhibition of Hsp90 by GA, 17-AAG, or radicicol reduced the protease activity of NS3 required to liberate it from the NS2/3 protein precursor, and caused NS3 proteasomal degradation [71, 72]. Interestingly, even though Hsp90 is required for the proteolytic activity of NS3, Hsp90 binds the helicase domain and not the protease domain. Accordingly, deletion of the helicase domain prevents the degradation of NS3 induced by Hsp90 inhibitors. NS5A also associates with Hsp90 and Hsp90 inhibition by GA results in a modest level of NS5A degradation [73]. As discussed above for the polymerase-Hsp90 interactions,

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the study of HCV encoded clients of Hsp90 also implicates specific Hsp90 co-chaperones in viral replications. Interestingly, the analysis of the NS5A-Hsp90 interactions revealed a novel type of membrane-anchored Hsp90 complex, where Hsp90 and NS5A are both associated with the proline-isomerase FKBP8 and a novel membrane-bound Hsp90 cofactor called human butyrate-induced transcript 1 (hB-ind1), which harbors a p23 homology domain [74]. These findings illustrate how the large collection of Hsp90 cofactors can direct individual viral proteins to specific cellular locales and functions. It is also noteworthy that replication of all positive-strand RNA viruses, such as HCV, occurs on intracellular membranes, raising the possibility that membrane-anchored Hsp90 cofactors, such as hB-ind1, target the chaperone to sites of viral replication and help assemble large replication complexes. Investigating the role of hB-ind1 and other Hsp90 cofactors in the replication of additional RNA viruses may lead to more specific therapeutic avenues to inhibit viral replication, since inhibition of specific Hsp90-cochaperone interactions could reduce pleiotropic adverse effects on the protein homeostasis of uninfected cells.

5. Viral structural proteins as Hsp90 clients

Viral structural proteins assemble to form the viral capsid, a complex protein structure that encapsulates the viral genome, protecting it from degradation and releassing it upon re-infection of a new cell. Viral capsids can be comprised of one or more proteins and can contain over 1000 subunits. These structures must be sufficiently stable both within the host and outside to withstand harsh environmental conditions, such as low pH and high temperature, and yet they must also disassemble readily upon entry into cells to deliver the viral genome. Therefore, the complexity of viral capsids is likely to be especially demanding on the cellular protein folding and assembly machinery.

Hsp90 is known to facilitate folding of several viral structural proteins. Hsp90 is required for replication of Picornaviridae family members poliovirus, rhinovirus, and coxsackievirus [33]. Mechanistic analyses of poliovirus and rhinovirus replication traced the Hsp90 requirement to a role in the maturation of the viral capsid proteins [33] (Fig. 1C). The picornavirus capsid consists of 60 copies of each of four subunits, which are generated by the cleavage of the P1 precursor protein by a viral encoded protease. Hsp90, in combination with the p23 cochaperone, was found to interact with P1 and to be required for the cleavage of P1 into the capsid subunits by the viral protease. Interestingly, Hsp90 did not interact with any other viral proteins and its inhibition did not affect the cleavage of virus encoded precursor proteins that are similarly cleaved by the same viral protease. Hence, as protease activity is not diminished with other viral proteins, it is likely that Hsp90 is required for inducing a P1 confirmation that enables recognition and/or cleavage by the protease [33]. Moreover, following cleavage, Hsp90 is no longer associated with the mature, cleaved capsid subunits, suggesting Hsp90 recognizes a state of P1 that is lost following cleavage. Since the Picornaviridae family is the largest viral family and both capsid structure and maturation pathway are conserved within this family, the wealth of sequence and structural information make P1 an interesting model for studying Hsp90-substrate interactions. It is interesting to note, however, that hepatitis A replication is not sensitive to Hsp90 inhibition; this hepatovirus is distinguished from other Picornaviridae in its extremely inefficient translation and replication kinetics, and may accordingly employ alternative strategies for P1 maturation [75].

The reovirus attachment protein σ1 also requires Hsp90 for folding. The σ1 protein forms a homotrimeric complex that decorates the viral capsid and mediates binding to host cell receptors [31, 32]. The folding of the σ1 protein occurs in a stepwise manner: upon synthesis on polysomes the emerging nascent chains begin to trimerize co-translationally, forming a folded, trimeric N-terminal domain; subsequently to completion of translation the

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C-terminal domain folds and trimerizes post-translationally [76, 77]. Notably, Hsp90 is essential for the post-translational folding step of the C-termini [78]. Accordingly, inhibition of Hsp90 blocks folding of the C-termini but does not affect trimerization of the N-terminal domain. Hsp90 recognizes a conformation that is lost upon folding and does interact with fully assembled σ1 trimers. Strikingly, translation of σ1 leads to Hsp90 phosphorylation, which in turn blocks the Hsp90-σ1 interaction, suggesting a possible novel feedback mechanism to release Hsp90 upon σ1 folding [79].

During vaccinia virus infection, inhibition of Hsp90 by GA or novobiocin reduces viral DNA replication by specifically inhibiting intermediate and late gene expression but not early gene expression [80, 81]. Hsp90 interacts with the 4a core protein of vaccinia virus, implicating Hsp90 in the conformational maturation of the vaccinia capsid. Furthermore, Hsp90 only colocalizes with the viral core protein 4a early during infection but not at later stages, suggesting a transient role for Hsp90 in virion morphogenesis [34]. Since vaccinia virus encodes over 250 proteins, it remains likely that Hsp90 and other chaperones play additional roles in the replication of this virus.

While all the above findings illustrate to a role of Hsp90 in viral capsid folding and assembly, it is also possible that Hsp90 associates with viral capsids at an early entry step such as penetration or uncoating, in which capsids must be transported in the cell and disassembled. This has in fact been suggested for Hepatitis E virus (HEV), where Hsp90 appears important for intracellular transport of the incoming viral particle [82].

6. Cellular factors associated with viral propagation, pathogenesis and the immune system

In addition to a direct interaction with viral proteins that mediates their folding, assembly and activity Hsp90 can also facilitate or modulate infection through regulation of host processes and cellular Hsp90 client proteins. The examples below illustrate the plurality of levels at which Hsp90 regulates viral infection. First, Hsp90 was reported to be present on the surface of some cells [83]. While the actual mechanism by which a cytosolic chaperone reaches the cell surface is not fully clear, plasma membrane associated Hsp90 and Hsp70 have been observed in both stressed and antigen-presenting cells [84]. Such surface exposed Hsp90 is proposed to play a role in the internalization of both dengue virus (DENV) and infectious bursal disease virus (IBDV) [85, 86]. In the case of dengue virus, the cell-surface Hsp90 is proposed to interact with an unknown viral receptor complex [86]. Addition of anti-Hsp90α or Hsp70 antibodies to the extracellular medium inhibits virus infection in monocytes and macrophages but does not effect dengue virus propagation in liver cells, suggesting a cell type specific requirement for plasma membrane localized Hsp90 [85]. Moreover, transient heat shock was shown to increase cell surface expression of Hsp90 and Hsp70 and also increase dengue virus infectivity [87]. Similarly to dengue virus, antibodies against Hsp90α were shown to inhibit the ability of IBDV to infect a cell line of chicken origin [88].

Hsp90 was implicated in supporting the efficient production of human immunodeficiency virus (HIV) proviral DNA by promoting the formation of a Cdk9/cyclin T1 complex, the components of the human positive transcription elongation factor P-TEFβ [89]. This complex phosphorylates cellular RNA polymerase II, resulting in its increased processivity. The HIV accessory protein Tat can then recruit P-TEFβ to the 5′ of HIV RNA and stimulate transcription of HIV proviral DNA. Hsp90, together with the co-chaperone Cdc37, were shown to bind Cdk9 and render it competent for binding cyclin T1. Inhibition of Hsp90 impaired formation of Cdk9/cyclin T1 complexes. While the relevance of this finding to

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HIV replication is unknown, Hsp90 inhibition by GA and coumermycin A1 was reported to reduce the replication of HIV [89, 90].

Viruses causing chronic infection might take advantage of chaperone machinery to modulate host cell viability to ensure persistence of the viral infection. Since Hsp90 modulates the activity of many signal transduction pathways and immune modulators, Hsp90 inhibitors may help clear such chronic viral infections. For instance, both the glycoprotein K1 and the viral FLIP protein (v-FLIP) of the gamma herpesvirus Kaposi sarcoma-associated herpesvirus (KSHV) activate the NF-kB pathway and block apoptosis to ensure survival of infected host cells [91, 92]. v-FLIP forms an IKK kinase complex with assistance of Hsp90 and Cdc37 [93]. Hsp90, together with other chaperones such as Hsp40, also interacts with K1 and prevents its proteasomal degradation [94]. Accordingly, inhibitors of Hsp90 block the activity of K1 and v-FLIP. As a result, NF-kB activation is impaired and apoptosis of infected cells is increased, helping clear the viral infection. Similar to KSHV, Epstein Bar virus (EBV) and human T-cell leukemia virus (HTLV)-1 also exploit Hsp90 function to promote survival of infected host cells thus prolonging the viral infection [95, 96].

7. Potential of Hsp90 inhibitors as antivirals

Viral infections are amongst the principal causes of human morbidity and mortality worldwide and impose severe economic burdens on society. Despite their importance, few effective antivirals currently exist and many medically and economically important viral infections lack any treatment (see [97] for review). One of the main hurdles to the development of effective antivirals is the rapid acquisition of drug resistance by viruses, which can limit the utility of even the most effective drugs and preclude their long-term use [98]. The high mutation rates, short replication times, large population sizes, and compact genomes enable viruses to rapidly acquire mutations conferring drug resistance, thus circumventing inhibition by antiviral drugs. The ability of viruses to develop drug resistance to antiviral compounds targeting viral proteins is enormous, since the drug target is under the replicative control of the virus and therefore escape mutations are easily generated. In the case of antivirals targeting cellular factors required for viral replication, acquisition of drug resistance has also been observed, although it is more difficult as the virus must either dispense with the affected function or evolve to employ an alternative cellular pathway. The lack of successful antiviral therapies for most viruses create a pressing need for identification of novel antivirals that do not elicit drug resistance.

Inhibition of Hsp90 constitutes a promising antiviral approach. First, as can be inferred from the multiplicity of different viruses requiring Hsp90 for replication, Hsp90 inhibitors are among the broadest-spectrum antivirals identified to date. Currently, Hsp90 inhibitors have been demonstrated to possess antiviral activity in tissue culture against picornaviruses (poliovirus, coxsackievirus, rhinovirus), influenza virus, paramyxoviruses (HPIV2, HPIV3, SV5, SV41), HCV, Ebola virus, vesicular stomatitis virus, La crosse virus, severe acquired respiratory syndrome (SARS), FHV, HIV, vaccinia virus, and herpes viruses (HSV1/2, HCMV, VZV). This property makes Hsp90 inhibitors particularly attractive antivirals for existing viral diseases lacking therapies and for rapid response to newly emerging viral diseases. Secondly, administration of Hsp90 inhibitors to infected animals was shown to reduce the replication of two different viruses, poliovirus and HCV, with little toxicity to the infected host [33, 99]. These experiments highlight the feasibility of using these inhibitors therapeutically in humans. Thirdly, Hsp90 inhibitors were demonstrated to be refractory to development of drug resistance. This was clearly highlighted by poliovirus experiments, as this virus has been demonstrated to gain drug resistance to all antivirals tested to date whether they target viral or host factors. However, when poliovirus was repeatedly treated with Hsp90 inhibitors, no drug resistance was observed despite extensive passaging of the

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virus in the presence of Hsp90 inhibitors in cultured cells. Similarly, no drug resistance was observed in viruses recovered from Hsp90 inhibitor treated mice. The lack of viral drug resistance to Hsp90 inhibitors suggests such an antiviral approach may be particularly useful for treatment of chronic viral infections and treatment of RNA virus infections for which drug resistance is most frequently observed.

The feasibility of transitioning Hsp90 inhibitors for antiviral use in humans is further supported by the fact that Hsp90 constitutes a highly druggable target. To date, numerous structurally diverse pharmacological inhibitors have been identified. The majority of these inhibit Hsp90 by blocking ATP binding, although inhibitors that block alternate sites on Hsp90 have also been identified. Moreover, Hsp90 activity is inhibited by acetylation [100] and histone deacetylase (HDAC) inhibitors have been reported to block Hsp90 activity [101-103]. The fact that Hsp90 inhibitors possess anti-cancer activity and cancer cells display ~100 fold hypersensitivity to Hsp90 inhibition relative to normal cells has led to the synthesis and human evaluation of many Hsp90 inhibitors, the most advanced of which are currently undergoing phase III clinical trials [104]. From data available to date, Hsp90 inhibitors appear to be reasonably well tolerated at concentrations sufficient for anticancer activity and show good tissue distribution. Importantly, for an acute viral infection, the course of inhibitor administration would be much shorter than those currently used for cancer, thus potentially lessening side effects observed in the much longer cancer therapies. Similarly, HDAC inhibitors are used in the clinic for cancer treatment. Together, these results show that Hsp90 inhibitors are safe for use in humans and could provide a viable antiviral therapeutic strategy.

Additional important consideration regarding the use of Hsp90 inhibitors as antivirals comes from the role of Hsp90 in inflammation, immunology, cellular antiviral defense pathways, and apoptosis [84, 105]. Hsp90 inhibitors have been shown to reduce inflammation [106-108]. This property may be useful during viral infections where pathology is in part due to inflammatory processes. Additional roles of Hsp90 in folding and activity of intracellular antiviral and inflammatory responses, such as interferon regulatory factor 3 (IRF3), TBK1, and the double-stranded RNA activated protein kinase (PKR) [109, 110], may play important roles in modulating the course of viral infection during treatment with Hsp90 inhibitors.

8. Future Directions and Perspectives

Proper folding is the essential ritual undergone by all proteins to achieve their correct structure and activity. Although it is clear that all viruses depend on the host chaperone machinery to fold their proteins, most of the mechanisms and cellular factors underlying viral protein homeostasis are still to be unveiled. Understanding this fascinating process is likely to reveal many important principles and aspects of chaperone function that are fundamental to not only viral biology but likely relevant to our understanding of normal protein homeostasis. With regards to broad-spectrum therapeutic avenues to combat viral infection, Hsp90 inhibitors hold considerable promise, particularly as new compounds with low toxicity and high specificity emerge. Characterization of specific co-chaperones involved in viral replication may provide additional targets to develop antivirals with fewer pleiotropic side effects. Nonetheless, the success of Hsp90 inhibitors for anticancer therapies, and the fact that many Hsp90 inhibitors are relatively well tolerated, bode well for harnessing them to be used as antivirals. To further support the development of Hsp90 inhibitors as antiviral therapeutics, future efforts should be aimed at careful evaluation of both the effectiveness and toxicity of Hsp90 inhibitors in animal models of infection for those viral infection where good animal models exist (e.g. influenza virus, coxsackievirus, HSV).

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Finally, it is important to note that Hsp90 does not work alone, and other chaperones, such as Hsp70, are also conscripted to facilitate viral protein folding and propagation. Hence, comprehensive understanding of chaperone use during viral infection will provide new insight into viral replication mechanisms and potential therapeutics.

Acknowledgments

We thank Dr. Raul Andino for useful discussions and Dr. Christine Livingston for comments on the manuscript. Work in the Frydman lab is supported by NIH grant GM56433. S.T. acknowledges support of grants-in-aid from the Naito Foundation and the Uehara Memorial Foundation.

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Highlights

- Viruses are intracellular pathogens responsible for a vast number of human diseases.
- The molecular chaperone Hsp90 is nearly universally required for viral protein replication.
- The unique characteristics of viral replication cause viruses to be hypersensitive to pharmacological inhibitors of Hsp90
- The near-universal requirement for Hsp90 provides a novel therapeutic avenue for development of broad-spectrum antiviral drugs.

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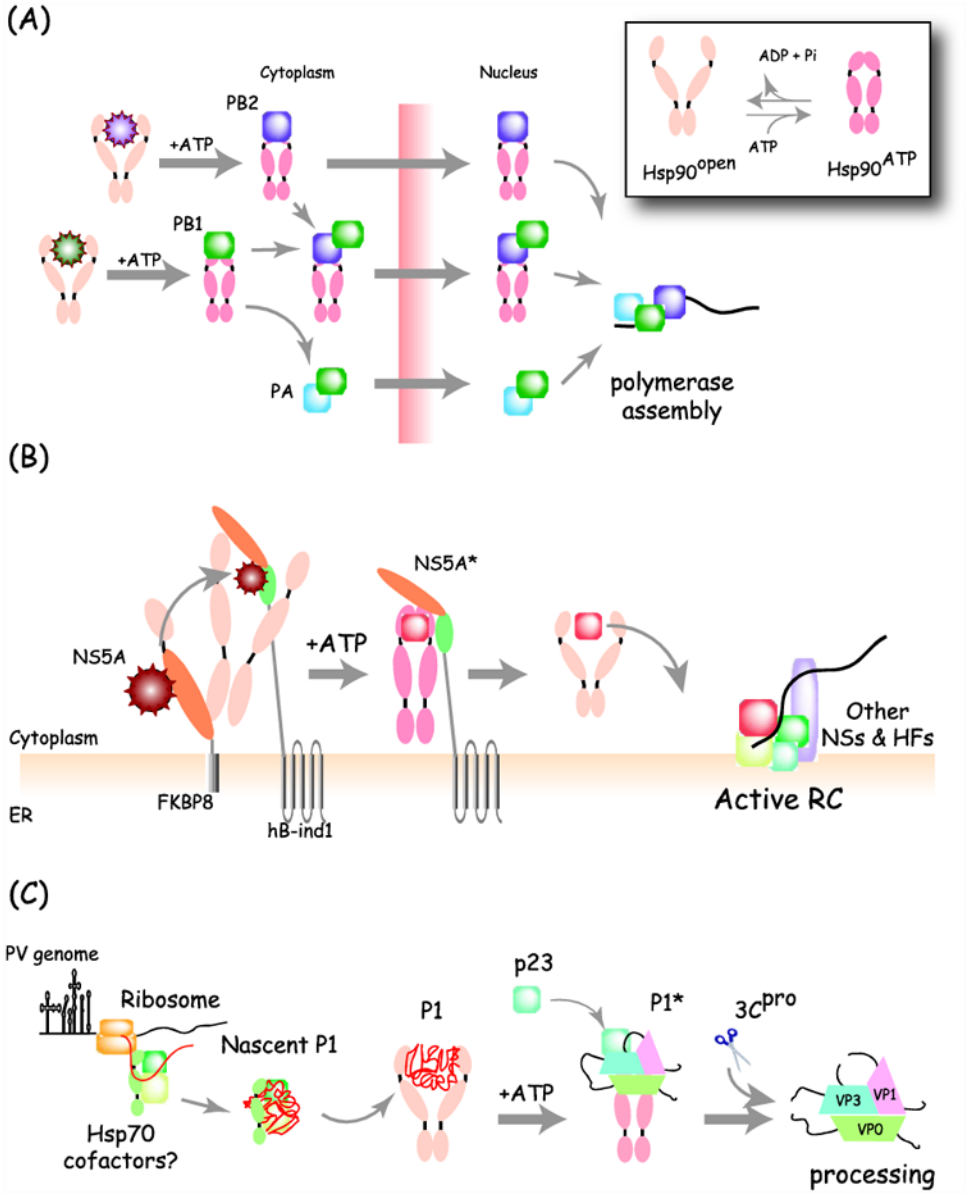

Figure 1. Distinct functions of Hsp90 during viral replication

(A) Hsp90-facilitates assembly of Influenza polymerase. Newly made influenza PB2 interacts with Hsp90 in the cytoplasm and the complex translocates into the nucleus. Another polymerase subunit PB1, which itself interacts with Hsp90, is also transported into the nucleus together with the PB2/Hsp90 complex. Once in the nucleus, Hsp90 dissociates as PB2 binds to PB1/PA to form the trimeric active polymerase. Inset: the two conformations of Hsp90 are regulated by its ATPase cycle. (B) Formation of a multi-chaperone complex with non-structural protein NS5A during HCV replication. HCV NS5A assembles into a complex containing Hsp90, FKBP8 and hB-ind1. Interaction with FKBP8 is required for NS5A entry into the Hsp90 cycle whereas hB-ind1 action stimulates dissociation from Hsp90 and correct NS5A folding (NS5A*), leading incorporation into an active replication complex (RC) containing other NS proteins and host factors (HFs). (C) Role of Hsp90 in picornavirus capsid maturation. Picornavirus capsid precursor poly-protein P1 binds associates with Hsp90, likely following an upstream interaction with Hsp70. Hsp90

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is required to fold P1 to a mature conformation (P1*) that is competent for proteolytic cleavage by the viral protease ($3C^{Pro}$) into the mature capsid protein. The processed capsid proteins no longer interact with Hsp90.

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Table. 1

Summary of the viruses and viral proteins currently known to require the Hsp90 chaperone

Viral family	virus	viral protein	Hsp90 dependent process	Additional factor	References
Herpesviridae	HSV1/HSV2	UL30	Polymerase localization	Hsp70, BAG3	[51, 52, 57]
	VZV	ORF29F	Localization of orf29	Hsp70, BAG3	[54]
	HCMV	-	Expression of immediate early protein IE2	PI3K	[53]
	EBV	KH	Apoptosis prevention	-	[96, 111]
		EBNA	Cell proliferation	-	[112]
	KSHV	KI	Apoptosis prevention	Hsp40	[94]
		v-FLIP	Apoptosis prevention	IKK, Cdc37	[93]
Polyomaviridae	SV40	LT	Stabilization of LT protein	-	[63]
Poxviridae	Vaccinia virus	4a core protein	Capsid assembly/ Virus gene expression	Hsp70	[34, 113]
Reoviridae	Reovirus	σ1	C' trimerization of σ1	Hsp70, p23	[76-79]
	Rotavirus	NSP3	Dimerization of NSP3	-	[66]
		-	-	PI3K	[70]
Birnaviridae	IBDV	VP2 (Capsid)	Virus Internalization	-	[88]
Picornaviridae	Poliovirus	P1 capsid protein	Cleavage of P1 into VP1, VP2 & VP3	p23	[33]
	Rhinovirus	P1 capsid protein	Cleavage of P1 into VP1, VP2 & VP3	p23	[33]
	Coxsackievirus	P1 capsid protein	Cleavage of P1 into VP1, VP2 & VP3	p23	[33]
Flaviviridae	HCV	NS3 Protease	Cleavage at NS2/3 junction, NS3 function	-	[71, 72, 99]
		NS5A	Replication complex formation/Genome replication	FKBP8/hB-ind1	[73, 74, 114, 115]
	DENV	Viral receptor	Virus Internalization	Hsp70/GRP78	[85, 86]
Arenaviridae	LCMV	NP	Antigen cross presentation	-	[116]
Nodaviridae	FHV	Protein A	Replication complex formation	-	[58, 59, 117]
Hepeviridae	HEV	Capsid	Intracellular transfer	-	[82]
Rhabdoviridae	VSV	L protein	Protein stabilization	-	[60]
Paramyxoviridae	HPIV2/3	L protein	Protein stabilization	-	[60]
	SV5/41	L protein	Protein stabilization	-	[60]
	Measles virus	-	Enhanced oncolytic activity	-	[118]
	Sendai virus	-	Innate immunity activation	TBK1	[110]
Bunyaviridae	La Crosse virus	L protein	Protein stabilization	-	[60]
Orthomyxoviridae	Influenza A Virus	PB1, PB2	Nuclear localization	-	[47]

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Viral family	virus	viral protein	Hsp90 dependent process	Additional factor	References
			vRNP complex formation	-	[48]
			RNA synthesis	-	[49]
Filoviridae	Ebola virus	-	Virus propagation	-	[119]
Retrovirus	HTLV1	tat	Transcription	-	[95]
	HIV1	tat	Transcription/Cell survival	-	[89, 90, 120]
Hepadnaviridae	DHBV	P protein	Reverse transcriptase priming	p23, Cdc37, Hsp40, Hsc70	[41, 43, 44, 121]
	HBV	P protein	Reverse transcriptase priming	Hsp70, Hsp40 Hop, P23	[43, 45, 46]

Abbreviations: HSV, herpes simplex virus; VZV, varicella zoster virus; HCMV, human cytomegalovirus; EBV, Epstein-Bar virus; KSHV, Kaposi sarcoma-associated herpesvirus; SV, simian virus; IBDV, infectious bursal disease virus; HCV, hepatitis C virus; DENV, dengue virus; LCMV, lymphocytic choriomeningitis virus; FHV, flock house virus; HEV, Hepatitis E virus; VSV, vesicular stomatitis virus; HPIV, human parainfluenza virus; vRNP, viral ribonucleoprotein complex; HTLV, human adult T cell leukemia virus; HIV, human immunodeficiency virus; DHBV, duck hepatitis B virus; HBV, hepatitis B virus.